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(54) Title: CHLAMYDIA PROTEINS AND THEIR USES

(57) Abstract

Certain *Chlamydia* proteins have been found to be infection-specific and to be associated primarily with the vegetative Reticulate Body form of *Chlamydia* rather than with the refractile Elementary Body form of *Chlamydia*. The invention includes a vaccine directed against the Reticulate Body form of *Chlamydia* comprising one or more infection-specific proteins, or fraction thereof; a method of using such a vaccine; a method of production of such a vaccine; a method for detection of infection-specific antibodies in a biological specimen; a method for detection of infection-specific antigens in a biological specimen and a method of using therapeutic agents specifically directed against infection-specific peptides, or the genes that code for such peptides, to treat chlamydial infection. The invention also includes the IncB, and IncC proteins of *C. psittaci*, and nucleotides encoding these proteins, and the TroA, TroB and p242 proteins of *C. trachomatis*, and the nucleotides that encode these polypeptides.

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CHLAMYDIA PROTEINS AND THEIR USES

I. FIELD OF THE INVENTION

The present invention relates to the detection of *Chlamydia* and to the diagnosis, treatment and prevention of *Chlamydia* infections in animals.

II. BACKGROUND

Chlamydiae are obligate intracellular bacterial pathogens with a unique biphasic life cycle. They appear as two distinct cellular types, a small dense cell or elementary body (EB) that is enclosed in a rigid bacterial cell wall, and a larger metabolically active reticulate body (RB). The EB is resistant to physical disruption and is infectious, whereas the RB is more fragile and only exists inside cells. The *Chlamydia* life cycle begins with the attachment of the EB form to the host cell which is followed by endocytosis into a nascent vacuole, also called an "inclusion membrane." After EB attachment and entry, replication of the EB form produces RB forms that continue to grow within the vacuole. By 72 hour post-infection, this growth phase is terminated when the RBs condense, and reorganize back to EBs. The lysis of the host cell results in release of EBs to infect new host cells. The difficulties in working with *Chlamydiae* center on the obligate intracellular requirement for growth and the fact that no adequate genetic engineering methods have been developed for this organism.

The genus *Chlamydia* includes two species that are primarily associated with human disease: *C. trachomatis* and *C. pneumoniae*. *C. trachomatis* causes trachoma, an eye disease that is the leading cause of preventable infectious blindness worldwide with an estimated 500 million cases of active trachoma worldwide. *C. trachomatis* also causes a sexually transmitted chlamydial disease which is very common worldwide. *C. trachomatis* also causes lymphogranuloma venereum, a debilitating systemic disease characterized by lymphatic gland swelling. The most serious sequelae of chlamydial genital infections of females include salpingitis, pelvic inflammatory disease, and ectopic pregnancy. In the US alone, it is estimated that over 4 million new sexually transmitted *C. trachomatis* infections occurred in 1990, leading to over four billion dollars in direct and indirect medical expenses. The World Health Organization estimates that 89 million new cases of genital *Chlamydia* occurred worldwide in 1995 (Peeling and Brunham, 1996).

C. pneumoniae causes respiratory diseases including so called walking pneumonia, a low-grade disease such that the infected person frequently fails to obtain treatment and remains in the community as an active, infectious carrier. *C. pneumoniae* is currently of interest because of its strong epidemiological association with coronary artery disease, and there is also some evidence to link it with multiple sclerosis.

Of the other disease-causing species of *Chlamydia*, *Chlamydia psittaci* and *Chlamydia pecorum* are primarily pathogens of wild and domestic animals, but these species may infect

humans accidentally. *C. psittaci* is acquired through respiratory droplet infection and is considered an occupational health hazard for bird fanciers and poultry workers.

There is tremendous interest in the identification of candidate antigens for protection against chlamydial disease. While a prior infection with *C. trachomatis* will protect against a subsequent challenge by the same strain, indicating a protective component that stimulates the host immune response, most serious chlamydial diseases are exacerbated by an overaggressive anti-chlamydial immune response. Antigens recognized in the context of an infection appear to elicit a protective response whereas immunization with purified, killed (EB form) *Chlamydia* results in an immunopathological response. Therefore for the purposes of vaccine development, one needs to find epitopes that confer protection, but do not contribute to pathology. It is an object of this invention to provide *Chlamydia* polypeptides for use as vaccines that induce a protective immune response without inducing the pathological response caused by the antigens associated with the EB form of *Chlamydia*. Such immunostimulatory peptides will be useful in the treatment, as well as in the diagnosis, detection and prevention of Chlamydial infections.

III. SUMMARY OF THE INVENTION

The present invention includes the use of *Chlamydia* proteins that show enhanced expression in the reticulate body (RB) stage relative to the elementary body (EB) stage of the *Chlamydia* life cycle. These proteins are not present at detectable levels in the EB form using current immunological techniques and are thus said to be "infection-specific." Certain of these infection-specific proteins are found in the inclusion membrane of the infected cell, and so have been termed "Inc" proteins. These include the IncA, IncB, and IncC proteins of *Chlamydia* as described in the present disclosure. The genes that encode the IncA, IncB and IncC proteins are referred to as *incA*, *incB* and *incC* respectively. Other proteins of *Chlamydia* described herein have also been shown by the inventors to be infection-specific, but are not known to be incorporated into the inclusion membrane; these include the p242, TroA, and TroB proteins. The TroA and TroB proteins have been so named because they resemble the Tro proteins of *Treponema pallidum*, which are thought to form part of an ABC transport system.

The inventors have shown that the infection-specific *Chlamydia* proteins of the disclosure are recognized by convalescent antisera (i.e., antisera taken from an animal that has recovered from a *Chlamydia* infection) but are not recognized by antisera against the killed EB form of *Chlamydia*. Thus, the proteins are expressed only during active chlamydial infection and are therefore useful as protective antigens. These infection-specific proteins may be used to confer a protective immune response without inducing a pathological effect. Additionally, immuno-fluorescence microscopy and immunoblotting with antisera demonstrated that the infection-specific proteins are present in *Chlamydia*-infected HeLa cells, but are undetectable in purified EBs and absent in uninfected HeLa cells.

Immunofluorescense microscopy reveals that IncA, IncB and IncC are localized to the inclusion membrane of infected HeLa cells. Reverse-transcription polymerase chain reactions (RT-PCR), northern hybridization data, and restriction analysis revealed that the *incB and incC* genes are closely linked and transcribed in an operon. RT-PCR, restriction analysis and sequential Southern hybridizations of *incA* then *incC* to the same filter provided evidence that *incA* is separated from the *incB* and *incC* operon by about 110 kb. The *C. trachomatis Tro* genes are not closely linked with the p242 gene.

The present invention includes the nucleotide and amino acid sequences for certain infection-specific proteins from *Chlamydia*. These proteins are p242, TroA, and TroB from *C. trachomatis*, and the IncB, and IncC proteins from *C. psittaci*. The scope of the invention includes fragments of these proteins that may be used in a vaccine preparation or that may be used in a method of detecting *Chlamydia* antibodies. Such fragments may be, for example, 5, 10, 15, 20, 25, or 30 contiguous amino acids in length. They may even encompass the entire protein.

More specifically, the present invention encompasses the purified infection-specific proteins having amino acid sequences as shown in SEQ ID NOS: 2, 4, 6, 10, and 12, amino acid sequences that differ from such sequences by one or more conservative amino acid substitutions, and amino acid sequences that show at least 75% sequence identity with such amino acid sequences.

Then invention also includes isolated nucleic acid molecules that encode a protein as described in the above paragraph, including isolated nucleic acid molecules with nucleotide sequences as shown in SEQ ID NOS: 1,3, 5, 9, and 11.

The present invention also includes a vaccine or immunostimulatory preparation directed against the reticulate body (RB) form of *Chlamydia* comprising one or more purified infection-specific peptides (or portions or fragments thereof, or peptides showing sequence similarity to a portion of such a peptide). Such peptide fragments may be, for example, 5, 10, 15, 20, 25, or 30 contiguous amino acids in length, of the sequence shown in SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, or 18. Peptides used in such a vaccine may even encompass the entire purified peptide of SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, or 18, a peptide that differs from such a peptide by one or more conservative amino acid substitutions, or a peptide having at least 75% sequence identity with such a peptide. Such vaccine preparations may contain one or more pharmaceutically acceptable excipients, adjuvants or diluents.

The invention additionally encompasses methods for making a vaccine, comprising combining a pharmaceutically acceptable excipient with a peptide described herein. Also included is a method of vaccination comprising administering a vaccine as described herein to a mammal.

The present invention also provides a method for the diagnostic use of the disclosed purified infection-specific peptides, for instance by use in a diagnostic assay to detect the presence of infection-specific antibodies in a medical specimen, in which antibodies bind to the *Chlamydia* peptide and indicate that the subject from which the specimen was removed was previously

exposed to *Chlamydia*. Such a method may comprise: (i) supplying a biological sample, such as blood from an animal, that is suspected to contain infection-specific anti-*Chlamydia* antibody, (ii) contacting the sample with at least one infection-specific *Chlamydia* peptide described herein, such that a reaction between the peptide and the infection-specific anti-*Chlamydia* antibody gives rise to a detectable effect, such as a chromogenic conversion; and (iii) detecting this detectable effect.

The present invention also provides a method of using antibodies that bind specifically with the disclosed proteins for detection of infection-specific *Chlamydia* antigen, indicating the presence of *Chlamydia* in the RB stage as distinct from the EB stage. For instance, the relevant infection-specific antibodies may be used to provide specific binding in an Enzyme Linked Immunosorbant Assay (ELISA) or other immunological assay wherein the antibody F_c portion is linked to a chromogenic, fluorescent or radioactive molecule and the F_{ab} portion specifically interacts with, and binds to, an infection-specific protein. Such a method may comprise: (i) supplying a biological sample from an animal suspected to contain an infection-specific *Chlamydia* antigen, and (ii) contacting the sample with at least one infection-specific anti-*Chlamydia* antibody, such that a reaction between the antibody and the infection-specific *Chlamydia* protein gives rise to a detectable effect; and (iii) detecting this detectable effect.

Other aspects of the present invention include the use of probes and primers derived from the nucleotide sequences that encode infection-specific peptides, to detect the presence of *Chlamydia* nucleic acids in medical specimens. Such probes and primers may be nucleotide fragments, of, for example, 15, 20, 25, 30 or 40 contiguous nucleotides of the sequence shown in SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, or 17.

An additional aspect of the invention is a method of treating a *Chlamydia* infection by directing a therapeutic agent against a specific target, where the target is chosen from an infection specific protein of *Chlamydia*, a gene that encodes an infection-specific protein of *Chlamydia*, and an RNA transcript that encodes an infection-specific protein of *Chlamydia*, wherein the therapeutic agent interacts with said target to affect a reduction in pathology.

These and other aspects of the invention will become more apparent from the following description.

IV. SEQUENCE LISTING

SEQ ID NO:1 shows a nucleic acid sequence encoding the p242 *C. trachomatis* protein, with deduced primary amino acid sequence also shown.

SEQ ID NO:2 shows the amino acid sequence of the p242 *C. trachomatis* protein.

SEQ ID NO:3 shows a nucleic acid sequence encoding the TroA *C. trachomatis* protein, with deduced primary amino acid sequence also shown.

SEQ ID NO:4 shows the amino acid sequence of the TroA *C. trachomatis* protein.

SEQ ID NO:5 shows a nucleic acid sequence encoding the TroB *C. trachomatis* protein, with deduced primary amino acid sequence also shown.

SEQ ID NO:6 shows the amino acid sequence of the TroB *C. trachomatis* protein.

SEQ ID NO:7 shows a nucleic acid sequence encoding the IncA *C. psittaci* protein, with deduced primary amino acid sequence also shown.

SEQ ID NO:8 shows the amino acid sequence of the IncA *C. psittaci* protein.

SEQ ID NO:9 shows a nucleic acid sequence encoding the IncB *C. psittaci* protein, with deduced primary amino acid sequence also shown.

SEQ ID NO:10 shows the amino acid sequence of the IncB *C. psittaci* protein.

SEQ ID NO:11 shows a nucleic acid sequence encoding the IncC *C. psittaci* protein, with deduced primary amino acid sequence also shown.

SEQ ID NO:12 shows the amino acid sequence of the IncC *C. psittaci* protein.

SEQ ID NO:13 shows a nucleic acid sequence encoding the IncA *C. trachomatis* protein, with deduced primary amino acid sequence also shown.

SEQ ID NO:14 shows the amino acid sequence of the IncA *C. trachomatis* protein.

SEQ ID NO:15 shows a nucleic acid sequence encoding the IncB *C. trachomatis* protein, with deduced primary amino acid sequence also shown.

SEQ ID NO:16 shows the amino acid sequence of the IncB *C. trachomatis* protein.

SEQ ID NO:17 shows a nucleic acid sequence encoding the IncC *C. trachomatis* protein, with deduced primary amino acid sequence also shown.

SEQ ID NO:18 shows the amino acid sequence of the IncC *C. trachomatis* protein.

SEQ ID NO:19 shows the upstream oligonucleotide used to amplify the *C. psittaci incC* ORF.

SEQ ID NO:20 shows the downstream oligonucleotide used to amplify the *C. psittaci incC* ORF.

SEQ ID NO:21 shows the upstream oligonucleotide used to amplify the *C. psittaci incB* ORF.

SEQ ID NO:22 shows the downstream oligonucleotide used to amplify the *C. psittaci incB* ORF.

SEQ ID NO:23 shows the upstream oligonucleotide used to amplify the *C. psittaci incA* ORF.

SEQ ID NO:24 shows the downstream oligonucleotide used to amplify the *C. psittaci incA* ORF.

V. DESCRIPTION OF THE INVENTION

A. DEFINITIONS

Particular terms and phrases used herein have the meanings set forth below.

"EB" refers to the Elementary Body, an environmentally refractile and largely metabolically dormant form of *Chlamydia* that is infectious and is presented as a small dense body enclosed by a bacterial cell wall.

"RB" refers to the Reticulate Body, a metabolically active form of *Chlamydia* that is not infectious, and exists only within a host cell, being very fragile, often branched, and appearing larger and less dense that the EB.

"Infection-specific" refers to a protein that shows enhanced expression in the RB form of *Chlamydia* compared to the EB form. Infection-specific proteins are not necessarily absent from the EB form, but they are significantly more common in the RB form than in the EB form.

"infection-specific antibody" is an antibody that binds specifically to an infection-specific protein.

"Biological sample" refers to any sample of biological origin including, but not limited to a blood sample, a plasma sample, a mucosal smear or a tissue sample.

"Isolated" An isolated nucleic acid has been substantially separated or purified away from other nucleic acid sequences in the cell of the organism in which the nucleic acid naturally occurs, i.e., other chromosomal and extrachromosomal DNA and RNA. The term "isolated" thus encompasses nucleic acids purified by standard nucleic acid purification methods. The term also embraces nucleic acids prepared by recombinant expression in a host cell as well as chemically synthesized nucleic acids.

"Probes" and "primers." Nucleic acid probes and primers may readily be prepared based on the nucleic acid sequences provided by this invention. A "probe" comprises an isolated nucleic acid attached to a detectable label or reporter molecule. Typical labels include radioactive isotopes, ligands, chemiluminescent agents, and enzymes.

"Primers" are short nucleic acids, typically DNA oligonucleotides 15 nucleotides or more in length, which are annealed to a complementary target DNA strand by nucleic acid hybridization to form a hybrid between the primer and the target DNA strand, then extended along the target DNA strand by a DNA polymerase enzyme. Primer pairs can be used for amplification of a nucleic acid sequence, e.g., by the polymerase chain reaction (PCR) or other nucleic-acid amplification methods known in the art.

Probes and primers as used in the present invention typically comprise at least 15 nucleotides of the nucleic acid sequences that are shown to encode infection-specific proteins. In order to enhance specificity, longer probes and primers may also be employed, such as probes and primers that comprise at least 20, 30 or 40 consecutive nucleotides of the disclosed nucleic acid sequences.

Methods for preparing and using probes and primers are well known in the art and are described in, for example Sambrook et al. (1989); Ausubel et al., (1987); and Innis et al., (1990). PCR primer pairs can be derived from a known sequence, for example, by using computer

programs intended for that purpose such as Primer (Version 0.5, 1991, Whitehead Institute for Biomedical Research, Cambridge, MA).

"Conservative amino acid substitutions" are those substitutions that, least interfere with the properties of the original protein, i.e., the structure and especially the function of the protein is conserved and not significantly changed by such substitutions. The table below shows amino acids which may be substituted for an original amino acid in a protein and which are regarded as conservative substitutions.

Original Residue	Conservative Substitution
Ala	Ser
Arg	Lys
Asn	gln, his
Asp	Glu
Cys	Ser
Gln	Asn
Glu	Asp
Gly	Pro
His	asn, gln
Ile	leu, val
Leu	ile, val
Lys	arg, gln, glu
Met	leu, ile
Phe	met, leu, tyr
Ser	Thr
Thr	Ser
Trp	Tyr
Tyr	trp, phe
Val	ile, leu

Conservative substitutions generally maintain (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain.

The substitutions which in general are expected to produce the greatest changes in protein properties will be non-conservative, for instance changes in which (a) a hydrophilic residue, e.g., seryl or threonyl, is substituted for (or by) a hydrophobic residue, e.g., leucyl, isoleucyl, phenylalanyl, valyl or alanyl; (b) a cysteine or proline is substituted for (or by) any other residue; (c) a residue having an electropositive side chain, e.g., lysyl, arginyl, or histadyl, is substituted for (or by) an electronegative residue, e.g., glutamyl or aspartyl; or (d) a residue having a bulky side chain, e.g., phenylalanine, is substituted for (or by) one not having a side chain, e.g., glycine.

"Sequence identity" The similarity between two nucleic acid sequences, or two amino acid sequences is expressed in terms of the level of sequence identity shared between the sequences. Sequence identity is typically expressed in terms of percentage identity; the higher the percentage, the more similar the two sequences are. Variants of naturally occurring infection-specific peptides useful in the present invention are typically characterized by possession of at least 50% sequence identity counted over the full length alignment with the amino acid sequence of a

naturally occurring infection-specific peptide when aligned using BLAST 2.0.1 (Altschul et al., 1997). For comparisons of amino acid sequences of greater than about 30 amino acids, the BLAST 2 analysis is employed using the blastp program set to default perameters (open gap = 11, extension gap = 1 penalty, gap x dropoff = 50, expect = 10, word size = 3, filter on), and using the default BLOSUM62 matrix (gap existence cost = 11, per residue gap cost = 1, lambda ratio = 0.85). When aligning short peptides (fewer than around 30 amino acids), the alignment should be performed using the Blast 2 sequences function, employing the PAM30 matrix (gap existence cost = 9, per residue gap cost = 1, lambda ratio = 0.87). Proteins with even greater similarity to the reference sequences will show increasing percentage identities when assessed by this method, such as at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, or at least 95% sequence identity. The NCBI Basic Local Alignment Search Tool (BLAST) (Altschul et al., 1990) is available from several sources, including the National Center for Biotechnology Information (NCBI, Bethesda, MD) and on the Internet, for use in connection with the sequence analysis programs blastp, blastn, blastx, tblastn and tblastx. It can be accessed at http//www.ncbi.nlm.nih.gov/BLAST/. A description of how to determine sequence identity using this program is available at http://www.ncbi.nlm.nih.gov/BLAST/blast_help.html.

Similarly, when comparing nucleotides, blastn may be used with default settings (rewards for match = 1, penalty for mismatch = -2, open gap = 5, extension gap = 2 penalty, gap x dropoff = 50, expect = 10, word size = 11, filter on), with the default BLOSUM62 matrix (as above). Variants of naturally occurring infection-specific nucleic acid sequences useful in the present invention are typically characterized by possession of at least 50% sequence identity counted over the full length alignment with the nucleic acid sequence of a naturally occurring infection-specific ORF when aligned using BLAST 2.0.1. Useful nucleic acids may show even greater percentage identity, and may, for example, possess at least 55%, at least 65%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% sequence identity naturally occurring infection-specific ORF.

"Operably linked" A first nucleic acid sequence is "operably" linked with a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Generally, operably linked DNA sequences are contiguous and, where necessary to join two protein coding regions, in the same reading frame.

"Recombinant" A recombinant nucleic acid is one that has a sequence that is not naturally occurring or has a sequence that is made by an artificial combination of two otherwise separated segments of sequence. This artificial combination is often accomplished by chemical synthesis or, more commonly, by the artificial manipulation of isolated segments of nucleic acids, e.g., by genetic engineering techniques.

"Stringent Conditions" Stringent conditions, in the context of nucleic acid hybridization, are sequence-dependent and are different under different environmental parameters. Generally, stringent conditions are selected to be about 5 degrees to 20 degrees lower than the thermal melting point (*Tm*) for the specific sequence at a defined ionic strength and pH. The *Tm* is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Conditions for nucleic acid hybridization and calculation of stringencies can be found in Sambrook et al. (1989), pages 9.49-9.55. Typical high stringency hybridization conditions (using radiolabeled probes to hybridize to nucleic acids immobilized on a nitrocellulose filter) may include, for example, wash conditions of 0.1 X SSC, 0.5% SDS at a wash temperature of 68°C.

When referring to a probe or primer, the term "specific for (a target sequence)" indicates that the probe or primer hybridizes under high-stringency conditions substantially only to the target sequence in a given sample comprising the target sequence.

"Purified" A purified peptide is a peptide that has been extracted from the cellular environment and separated from substantially all other cellular peptides. As used herein, the term peptide includes peptides, polypeptides and proteins. In certain embodiments, a purified peptide is a preparation in which the subject peptide comprises 50% or more of the protein content of the preparation. For certain uses, such as vaccine preparations, even greater purity may be preferable.

"Immunostimulatory peptide" as used herein refers to a peptide that is capable of stimulating a humoral or antibody-mediated immune response when inoculated into an animal.

"Vaccine" A vaccine is a composition containing at least one immunostimulatory peptide which may be inoculated into an animal with the intention of producing a protective immunological reaction against a certain antigen. The antigen to be protected against may be, for instance, an infectio-specific antigen of *Chlamydia*.

B. ISOLATION OF INFECTION SPECIFIC CHLAMYDIA POLPEPTIDES AND IDENTIFICATION OF GENES ENCODING THESE POLYPEPTIDES

1. ISOLATION OF IncA, IncB AND IncC

Bacterial strains. *Chlamydia* (C. *psittaci* strain GPIC or *C. trachomatis* LGV-434, ser. L2) was cultivated in HeLa 229 cells using standard methods (Caldwell et al., 1981). Purified *Chlamydiae* were obtained using Renografin (E. R. Squibb & Sons, Inc., Princeton, N.J.) density gradient centrifugation. *Escherichia coli* DH5α (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) was used as the host strain for transformations with recombinant DNA. *E. coli* XL1-Blue MRF' (Stratagene, La Jolla, Calif.) was used as the host strain for infection with lambda ZAPII phage vector. *E. coli* SOLR (Stratagene) was used as the host strain for infection with *in vivo* excised filamentous lambda ZAPII.

Antisera. MBP (Maltose Binding Protein)-Inc fusion proteins were used as antigens for the production of mono-specific antibody reagents in Hartley strain guinea-pigs. The protein was diluted to 100 $\mu g/ml^{-1}$ sterile saline and mixed with the Ribi Trivalent Adjuvant (Ribi Immunochem.). The antigen/adjuvant emulsion was administered to anaesthetized guinea-pigs using a procedure provided by the manufacturer. Sera were collected 14 days after secondary and tertiary immunizations. Control antisera were produced by immunizing guinea-pigs with adjuvant alone, or with adjuvant plus purified maltose-binding protein.

Convalescent guinea-pig antisera, antisera against live EBs, and antisera against formalin-fixed EBs were produced using standard methods (Rockey and Rosquist, 1994 and Rockey et al., 1995).

***C. psittaci* library construction and screening**. For the *inc*B and *inc*C genes, *C. psittaci* strain GPIC DNA was extracted using a genomic DNA extraction kit (Qiagen) with one modification; dithiothreitol (5mM) was added to the suspension buffer to assist EB lysis. DNA was partially digested with *Tsp*509I and ligated to *Eco*RI digested λ-ZAPII phage arms (Stratagene). The ligation was packaged in vitro with Gigapack extracts according to the manufacturer's instructions (Stratagene). Recombinant phage were plated on *E. coli* XL-1 Blue at densities of approximately 10^4 PFU/150-mm (diameter) plate. Following a nine hour incubation to allow development of the plaques, the plates were sequentially overlaid with nitrocellulose disks and the resulting lifts were processed for immunoblotting with convalescent antisera and antisera to fixed EBs. Of approximately 8,000 plaques, 18 had reactivity with the convalescent sera but not sera generated against EBs. One of these was subcloned into pBluescript SK(-) phagmid by *in vitro* excision in the *E. coli* SOLR strain (Stratagene) and designated pBS200-7.

For the *inc*A gene, genomic DNA from *C. psittaci* strain GPIC was partially digested with *Sau*3A, size-selected (2-8 kb) by electrophoresis through low-melting-temperature agarose, and blunt-ended with T4 DNA polymerase. This DNA was ligated to an *Eco*R1/*Not*1 adapter (Life Technologies), kinased, and ligated to *Eco*R1-digested Lambda ZAP II vector (Stratagene Cloning Systems). Recombinants were packaged (Lambda Gigapack Gold, Stratagene) and used to infect *E. coli* XL1-Blue (Stratagene). Plaques were allowed to develop for 4 h at 37°C. Nitrocellulose filters laden with 10 mM IPTG (US Biochemical Corp.) were placed onto the plaques and incubated for an additional 4 h at 37°C. These filters were removed and placed into a blocking solution consisting of PBS (150 mM NaCl, 10 mM $NaPO_4$, pH7.2) plus 0.1% Tween-20 (TPBS) and 2% BSA-TPBS. Filters were incubated for 1 h, rinsed twice in TPBS, and incubated overnight in convalescent-guinea-pig sera diluted 1:100 in BSA-TPBS. After three washes in TPBS, the filters were incubated for 1 h in ^{125}I-staphylococcal protein A (New England Nuclear) diluted to approx. 124 $nCiml^{-1}$ in BSA-TPBS. Filters were again washed three times in TPBS and positive plaques were detected by exposure of the dried filters to autoradiography film overnight at room temperature. Positive clones were picked and plaque-purified. pBluescript-SK- plasmids

containing the chlamydial genes of interest were recovered from the purified bacteriophage using ExAssist filamentous bacteriophages (Stratagene).

Identification of antigens recognized by convalescent antisera. Recombinant plaques were identified that showed reactivity with convalescent (anti-RB) antisera, but not with anti-EB serum. The purified recombinant phage were converted into pBluescriptII SK plasmid by *in vivo* excision and recircularization and these recombinant DNAs were used to transform *E. coli*. SDS-PAGE and immunoblot analysis of lysates of these recombinant *E. coli* showed that each expressed one or more proteins that reacted with convalescent antisera but not with the EB serum.

DNA Cloning and fusion protein production. The plasmid pJC2 contains a 5.0 kb *Eco*RI GPIC genomic fragment cloned into the pZEro2.1 vector (Invitrogen). To construct pJC2, the *incC* ORF sequence was ^{32}P-radiolabeled using random priming (Gibco-BRL) and used to probe *Eco*RI cut GPIC genomic DNA fragments separated by agarose gel electrophoresis. Fragments in the size range of the positive signal were excised from the gel and purified by Gene-Clean (Bio101). The gel-purified fragments were used in a ligation along with *Eco*RI-digested pZEro2.1. Kanamycin resistant colonies were screened by colony hybridization with radiolabeled *incC*.

MBP fusions of the five ORFs present in pJC2 were produced using the pMAL-C2 vector (New England Biolabs). The reading frame of *incC*, with the exception of the first four codons, was amplified using *Pwo* polymerase (Boehringer Mannheim) and pBS200-7 as the template. The upstream and downstream oligonucleotides for this amplification were

5'-AGAACCGATTTAACTCCAGGCG-3' (SEQ ID NO: 19) and

5'-GCGCGGATCCTTAATGTCCGGTAGGCCTAG-3' (SEQ ID NO: 20), respectively. The vector was digested with *Xmn*I and *Bam*HI, and the amplication product was digested with *Bam*HI. Ligation of these products resulted in an in-frame fusion between the *malE* gene in the vector and the *incC* reading frame from pBS200-7. The stop codon for this construction is provided by the insert. Following ligation, the products were transformed into *E.coli* strain HD5□. The resulting fusion protein (MBP/IncC) was overexpressed and purified by maltose affinity chromatography using an amylose resin supplied by New England Biolabs.

The same approach was used for production of the MBP/IncB fusion protein. The sequence encoding the N-terminal 101 amino acids of the IncB ORF was PCR amplified using the oligonucleotides

5'-ATGTCAACAACACCAGCATCTTC-3' (SEQ ID NO: 21) and

5'-GCGCGGATCCTTAATTAGTGCCTTCTGGATTAGG-3' (SEQ ID NO: 22).

The purified MBP/IncB and MBP/IncC fusion proteins were used as antigen for the production of monospecific antibody in Hartley strain guinea-pigs by standard methods (Rockey et al., 1995). Inserts in each construct were confirmed by DNA sequencing.

For IncA, a maltose-binding protein/IncA fusion protein was produced using the pMAL-C2 vector system from New England Biolabs. The reading frame of *incA* shown in Fig.1, with the exception of the initiator ATG, the *inc*A ORF was amplified using Vent DNA polymerase (New England Biolabs) and plasmid pGP17 as template. The upstream and downstream oligonucleotides for this amplification were

5'-CGCAGTACTGTATCCACAGACAAC-3' (SEQ ID NO: 23) and

5'-GTCGGATCCGAGAAACTCTCCATGCC-3' (SEQ ID NO: 24), respectively. The vector was digested with *Xmn*I and *Bam*HI, and the amplification product was digested with *Sca*I and *Bam*HI. Ligation of these products resulted in an in-frame fusion between the *malE* gene in the vector and the *incA* reading frame from pGP17. The stop codon for this construction is provided by the insert. Following ligation, the products were transformed into *E. coli* strain DH5□. The resulting fusion protein (MBP/IncA) was overexpressed and purified by maltose affinity chromatography using amylose resin (New England Biolabs).

MBP/IncA was used as antigen for the production of mono-specific antibody reagents in Hartley strain guinea-pigs.

DNA sequencing and sequence analysis. The pBS200-7 and pJC2 genomic clones as well as the MBP fusions were sequenced with the *Taq* DyeDeoxy Terminator Cycle Sequencing Kit (Perkin Elmer/Applied Biosystems Division). Several internal primers were designed to sequence further into the cloned inserts. Sequence assembly was performed using AssemblyLIGN software and sequence analysis was performed with MacVector software (International Biotechnologies Incorporated). Hydrophilicity profiles were determined using the Kyte-Doolittle scale (Kyte and Doolittle, 1982) with a window of 7. Deduced amino acid sequences were compared with the database using the BLAST program (on default settings) available from the National Center for Biotechnology Information on the world wide web. The entire nucleotide sequence of the pJC2 insert was deposited in the GenBank/EMBL Nucleotide Sequence Data Library, under accession number AF017105.

For *incA*, nucleotide sequencing was conducted using the Sequences system (US Biochemical) with the M13 forward and reverse primers, and internal primers synthesized on an Milligen/Biosearch Cyclone Plus DNA synthesizer. Computer analyses were conducted using the MacVector Sequence Analysis Software (International Biotechnologies Incorporated). Hydrophilicity profiles were determined using the Kyte-Doolittle scale (Kyte and Doolittle, 1982) with a window of 7. Secondary-structure predictions were generated using a combination of the Chou-Fasman and Robson-Garnier methods (Robson and Suzuki, 1976; Chou and Fasman, 1978). Deduced amino acid sequences were compared with those in the EMBL and GenBank databases using the BLASTP program available from the National Center for Biotechnology Information.

Electrophoresis and immunoblotting. Polyacrylamide gel electrophoresis (PAGE) was conducted using standard methods (Rockey and Rosquist, 1994). Immunoblotting was performed using standard methods (Rockey et al., 1995).

Immunofluorescence studies. *Chlamydiae* grown in HeLa cells on sterile glass coverslips were fixed for microscopy one of two ways. Cells were either incubated in methanol for 5 minutes, or in the combination fixative periodate-lysine-paraformaldephyde (PLP) for three hours at room temperature followed by permeabilization with 0.05% saponin (Brown and Farquhar, 1989). Immunostaining of the fixed coverslips was performed according to standard methods (Rockey et al., 1995) and visualized under a Nikon Microphot FXA microscope using the 63x objective and oil immersion.

RT-PCR analysis. RNA for RT-PCR analysis was extracted from approximately 2 x 10^{14} *C. psittaci*-infected cells. A Qiagen column was used for extraction and purification according to the manufacturer's instructions (Qiagen). RQ1 RNase DNase (Promega) was used to ensure removal of contaminating genomic DNA. cDNA was prepared by incubating 1.5 µg of RNA, 2.5 µM of the reverse oligonucleotide primer, and AMV reverse transcriptase (Promega) for 1 hour at 42°C in sodium pyrophosphate buffer, according to the manufacturer's instructions. PCR reactions were carried out using 1 µl of the cDNA reaction, 1.25 µM of each oligonucleotide primer, and *Pwo* polymerase (Boehringer Mannheim). Each RT-PCR reaction was accompanied by a positive control reaction that utilized the same primer set and 10 ng of *C. psittaci* genomic DNA, and a negative control reaction in which 1 µl of the same RNA preparation was used as template in the PCR reaction. A control primer set located within the *incC* gene was also used as an RT-PCR control.

Identification of *inc*A, *inc*B and *inc*C genes of *C. trachomatis*. The nucleotide sequence information obtained for the *inc*A, *inc*B and *inc*C of *C. psittaci* (above) was used, with standard methods, to identify the *inc* gene orthologues of *C. trachomatis*. Probes were made that corresponded to the 3' and 5' ends of the *C. psittaci inc* open reading frames. Standard PCR amplification (as above) was used, with the *C. trachomatis* genome as a template, to amplify the corresponding *C. trachomatis* nucleotide sequence. The amplified DNA was then sequenced, using standard methods.

2. ISOLATION OF p242, TroA AND TroB

Bacterial strains. *C. trachomatis* LGV-434, serotype L2, was cultivated in HeLa 229 cells using standard methods (Caldwell et al., 1981). Purified *chlamydiae* were obtained using Renografin (E. R. Squibb & Sons, Inc., Princton, N.J.) density gradient centrifugation (Hackstadt et al., 1992). *Escherichia coli* DH5α (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) was used as the host strain for transformations with recombinant DNA. *E. coli* XL1-Blue MRF' (Stratagene, La Jolla, Calif.) was used as the host strain for infection with lambda ZAPII phage

vector. *E. coli* SOLR (Stratagene) was used as the host strain for infection with *in vivo* excised filamentous lambda ZAPII.

Antisera. Two Cynomolgus monkeys (*Macaca fasicularis*) were anaesthetized and infected urethrally with *C. trachomatis* EBs. Each monkey was infected twice and allowed to recover between infections. Symptoms of infection were monitored over time. Antisera from infected monkeys were tested for reactivity to *Chlamydia* by ELISA (Su et al., 1990).

Sera were collected every two weeks and anti-chlamydial titers were determined. These animals showed mild clinical signs of disease which cleared spontaneously. A second challenge was then administered. Sera were collected from these animals and used to probe a *C. trachomatis* expression library as discussed below. As a control, Guinea Pigs were immunized with killed *C. trachomatis* of the EB form. Sera from these animals were obtained and also used to probe the *C. trachomatis* expression library.

***C. trachomatis* library construction and immunoscreening.** A *C. trachomatis* genomic library was constructed with the lambda ZAPII vector as described above for *C. psittaci*. Approximately 15,000 plaques were plated, transferred to nitrocellulose filters (Schleicher and Schuell, Keene, N.H.) in duplicate, and probed with the monkey convalescent antiserum and with Guinea Pig serum against killed EBs (Bannantine et al., 1998). Plaques that reacted only with the monkey convalescent antisera were selected for further study.

Identification of antigens recognized by convalescent antisera. Four positive recombinant plaques were identified that showed reactivity with convalescent antisera but not with anti-EB serum. The purified recombinant phage were converted into pBluescriptII SK plasmid by *in vivo* excision and recircularization and these recombinant DNAs (pCt1, pCt2, pCt3 and pCt4) were used to transform *E. coli*. SDS-PAGE and immunoblot analysis of lysates of these recombinant *E. coli* showed that each expressed one or more proteins that reacted with convalescent (anti-RB) antisera but not with the anti-EB antiserum. Two of the recombinants clones, pCt2 and pCt3, expressed an identical 19.9 kDa protein (p242). The pCt4 recombinant expressed two different proteins of approximately 32 kDa each that are strongly recognized by convalescent antisera (TroA and TroB).

C. SEQUENCE ANALYSIS

Sequence analysis of pCt1, 2, and 3 revealed overlapping inserts with only one open reading frame (ORF) common in all three. This ORF encodes an approximately 19.9 kDa protein (p242) that shows no similarity to other known proteins. The nucleotide sequence encoding *C. trachomatis* p242, and the amino acid sequence of the protein are shown in SEQ ID NOS:1 and 2, respectively.

The insert in pCt4 contains two complete ORFs which code for two proteins, each of approximately 32kDa (TroA and TroB) that show some homology with proteins from *Treponema*

pallidum. The nucleotide sequences encoding the 32 kDa proteins (TroA and TroB) and the amino acid sequences of these proteins are shown in SEQ ID NOS: 3, 4, 5, and 6.

D. EMBODIMENTS OF THE INVENTION

The present invention includes the nucleotide and amino acid sequences for certain infection-specific proteins from *Chlamydia*. These proteins are p242, TroA, and TroB from *C. trachomatis*, and the IncB, and IncC proteins from *C. psittaci*. The scope of the invention includes fragments of these proteins that may be used in a vaccine preparation or that may be used in a method of detecting *Chlamydia* antibodies. Such fragments may be, for example, 5, 10, 15, 20, 25, or 30 contiguous amino acids in length, or may even encompass the entire protein.

The present invention also encompasses the use of infection-specific proteins of *Chlamydia*, and the use of nucleotides encoding such proteins. Infection-specific proteins include the IncA, IncB and IncC proteins of *C. psittaci*, the IncA, IncB and IncC proteins of *C. trachomatis*, and the TroA, TroB, and p242 proteins of *C.* trachomatis. The inventors have shown that these proteins are infection-specific by using immunological techniques such as immuno-fluorescence microscopy and immunoblotting.

The present invention includes a vaccine against chlamydial infections comprising infection-specific proteins or fragments of these proteins or proteins that are homologous or show substantial sequence similarity to these proteins. In one embodiment, one or more purified infection-specific proteins may be mixed with a pharmaceutically acceptable excipient to produce a vaccine that stimulates a protective immunological response in an animal. In one embodiment the vaccine may be administered intra-muscularly or sub-cutaneously or intravenously. In another embodiment, the vaccine may be administered by inoculation into or onto the mucous membranes of the subject animal. For example, the vaccine may be administered urethrally or genitally as a liquid or in the form of a pessary. In another embodiment, it may be administered to the mucosa of the lungs as a spray or vapor suspension.

Since at least three amino acids are required to produce an antigenic epitope, the vaccine should comprise at least three consecutive amino acids, preferably at least five consecutive amino acids, and may comprise at least 10, 15, 25, 30, 40, or 45 consecutive amino acids of the infection-specific proteins as shown in SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, and 18.

The vaccine of the invention may be used to inoculate potential animal targets of any of the chlamydial diseases including those caused by *C. psittaci, C. trachomatis, C. pneumoniae* or *C. pecorum*. Indeed the vaccine of the invention may be used to inoculate animals against any disease that shows immunological cross-protection as a result of exposure to infection-specific *Chlamydia* antigen.

Vaccines of the present invention can include effective amounts of immunological adjuvants known to enhance an immune response (e.g., alum). The protein or polypeptide is present in the vaccine in an amount sufficient to induce a protective immune response whether

through humoral or cell mediated pathways or through both. Such a response protects the immunized animal against chlamydial infections specifically by raising an immune response against the Reticulate Body form of *Chlamydia*. Protective antibodies may be elicited by a series of two or three doses of the antigenic vaccine given about two weeks apart.

The present invention also teaches a method of making a vaccine against chlamydial infections. The method of making the vaccine comprises providing a pure (or substantially pure) infection-specific chlamydial peptide or portion thereof, and mixing the peptide with a pharmacologically acceptable excipient or adjuvant. Adjuvants may include commonly used compounds such as alum. Additionally, the vaccines may be formulated using a peptide according to the present invention together with a pharmaceutically acceptable excipient such as water, saline, dextrose and glycerol. The vaccines may also include auxiliary substances such as emulsifying agents and pH buffers. Doses of the vaccine administered will vary depending on the antigenicity of the particular peptide or peptide combination employed in the vaccine and characteristics of the animal or human patient to be vaccinated.

The infection-specific vaccine of the invention is directed towards not only *C. psittaci*, but against all forms of *Chlamydia* including *C. pneumoniae*, *C. trachomatis* and *C. pecorum*, and the vaccine may comprise not just peptides derived from *C. psittaci*, but also orthologous peptides and fragments of such orthologous peptides from other species of *Chlamydia* and peptides that are substantially similar to such peptides.

The present invention also teaches a method of vaccination comprising administering a vaccine formulated as described above to an animal either intravenously, intramuscularly, subcutaneously, by inhalation of a vapor or mist, or by inoculation in the form of a liquid, spray, ointment, pessary or pill into or onto the mucous membranes of the mouth, nose, lungs or urogenital tract or colon.

The methods of the invention may be practiced equally with human or non-human animal subjects.

The present invention also teaches a method of detecting *Chlamydia* infection-specific proteins produced by the Reticulate Body form of the organism. In this embodiment, antibodies raised to the infection-specific proteins are used in an immunological assay such as an Enzyme Linked Immunosorbant Assay or Biotin-Avidin assay or a radioimmunoassay or any other assay wherein specific antibodies are used to recognize a specific protein. Such assays may be used to detect both the quantity of proteins present and also the specificity of binding of such proteins. In such an assay, antibodies have attached to them, usually at the *Fc* portion, a detectable label, such as an enzyme, fluorescent marker, a radioactive marker or a Biotin-Avidin system marker that allows detection. A biological sample is provided from an animal that has been putatively exposed to *Chlamydia*. Such a sample may be, for example, whole blood, serum, tissue, saliva or a mucosal secretion. The sample is then contacted with the labeled antibody and specific binding, if any, is detected. Other methods of using infection-specific antibodies to detect infection-specific

antigens that are present in cells or tissues include immunofluorescense, indirect-immunofluorescense and immunohistochemistry. In immunofluorescense, a fluorescent dye is bound directly to the antibody. In indirect-immunofluorescence, the dye is bound to an anti-immunoglobulin. Specific binding occurs between antigen and bound antibody is detected by virtue of flourescent emissions from the dye moiety. This technique would be particularly useful, for instance, for detection of *Chlamydia* antigen present on a urogenital mucosal smear.

Other techniques, such as competitive inhibition assays may also be used to assay for antigen, and one of ordinary skill in the art will readily appreciate that the precise methods disclosed may be modified or varied without departing from the subject or spirit of the invention taught herein.

The present invention also teaches a method of detection of *Chlamydia* infection-specific antibodies made against the Reticulate Body. In this embodiment a sample is provided from an animal putatively exposed to *Chlamydia* to determine whether the sample contains infection-specific antibodies. Such a sample may be, for example, whole blood, serum, tissue, saliva or a mucosal secretion. This sample is contacted with infection-specific antigens such that the amount and specificity of binding of the antibody may be measured by its binding to a specific antigen. Many techniques are commonly known in the art for the detection and quantification of antigen. Most commonly, the purified antigen will be bound to a substrate, the antibody of the sample will bind via its *Fab* portion to this antigen, the substrate will then be washed and a second, labeled antibody will then be added which will bind to the *Fc* portion of the antibody that is the subject of the assay. The second, labeled antibody will be species specific, i.e., if the serum is from a human, the second, labeled antibody will be anti-human-IgG antibody. The specimen will then be washed and the amount of the second, labeled antibody that has been bound will be detected and quantified by standard methods.

The present invention also teaches a method of treating a *Chlamydial* infection by directing a therapeutic agent against a specific target, such as: (i) an infection-specific protein of *Chlamydia*, (ii) a gene that encodes an infection-specific protein of *Chlamydia* and (iii) an RNA transcript that encodes an infection-specific protein of *Chlamydia*, wherein said therapeutic agent interacts with said target to affect a reduction in pathology.

For example, the present invention teaches a method of treating chlamydial infection wherein antisense technology is used to prevent the expression of infection-specific genes, thereby preventing the pathologies associated these proteins and preventing reproduction of the RB phase of *Chlamydia*. In this embodiment, RNA molecules complementary to transcripts of infection specific genes are introduced into the host cells that contain *Chlamydia*, and by binding to the mRNA transcripts of the infection-specific genes, prevent translation and therefore expression of the infection-specific proteins that are associated with pathogenesis.

The invention may be practiced to produce a vaccine against any species of *Chlamydia*, including *C. psittaci*, *C. pecorum*, *C. trachomatis and C. pneumoniae*.

The following examples illustrate various embodiments of the invention.

EXAMPLE 1: Homologous Sequences

The DNA and protein sequences discussed herein are shown in SEQ ID NOS:1-18. These sequences refer to infection-specific proteins and to the DNA sequences that encode these proteins. Although these sequences are from *C. psittaci* and *C. trachomatis*, it would be equally possible to substitute in the present invention, the orthologs of these sequences from other *Chlamydia* species such as *C. pecorum* and *C. pneumoniae*.

Such orthologous sequences may be obtained from the appropriate organisms by isolation of the genome of the organism, digestion with restriction enzymes, separation of restriction fragments by electrophoresis and purification of these fragments and selection of fragments of appropriate size. Identity of the fragments can be confirmed by dot-blot and by standard DNA sequencing techniques. The orthologous sequences in different *Chlamydia* species may also be found by selection of appropriate PCR primers (selected from appropriate regions flanking the *Chlamydia* gene of interest), and the use of these primers in a PCR reaction, using the genome of the particular species of *Chlamydia* of interest as a template, to amplify the ortholog of interest. Such PCR primers would be selected from the flanking regions to allow specific amplification of the target gene. The fragments so obtained could then be run on a gel to check size and sequenced and compared against the known sequences to determine sequence identity.

The degree of sequence identity between the infection-specific genes of *C. psittaci* or *C. trachomatis* and their orthologs from *C. pecorum* and *C. pneumoniae,* may be determined by comparing sequences using the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) as described herein.

Orthologues of interest infection-specific proteins are characterized by possession of at least 50% or greater sequence identity counted over the full length alignment with one of the disclosed amino acid sequences of the *C. psittaci* or *C. trachomatis* infection-specific proteins using gapped blastp set to default parameters (described herein).

EXAMPLE 2: Heterologous Expression of Infection-Specific Antigens

Methods for expressing large amounts of protein from a cloned gene introduced into *Escherichia coli* (*E. coli*) may be utilized for the purification of the *Chlamydia* peptides. Methods and plasmid vectors for producing fusion proteins and intact native proteins in bacteria are well known and are described in Sambrook et al. (1989). Such fusion proteins may be made in large amounts, are relatively simple to purify, and can be used to produce antibodies. Native proteins can be produced in bacteria by placing a strong, regulated promoter and an efficient ribosome binding site upstream of the cloned gene. If low levels of protein are produced, additional steps

may be taken to increase protein production; if high levels of protein are produced, purification is relatively easy.

Often, proteins expressed at high levels are found in insoluble inclusion bodies. Methods for extracting proteins from these aggregates are described in chapter 17 of Sambrook et al. (1989). Vector systems suitable for the expression of *lacZ* fusion genes include the pUC series of vectors (Ruther et al. (1983)), pEX1-3 (Stanley and Luzio (1984)) and pMR100 (Gray et al. (1982)). Vectors suitable for the production of intact native proteins include pKC30 (Shimatake and Rosenberg (1981)), pKK177-3 (Amann and Brosius (1985)) and pET-3 (Studiar and Moffatt (1986)).

Fusion proteins may be isolated from protein gels, lyophilized, ground into a powder and used as antigen preparations.

Mammalian or other eukaryotic host cells, such as those of yeast, filamentous fungi, plant, insect, amphibian or avian species, may also be used for protein expression, as is well known in the art. Examples of commonly used mammalian host cell lines are VERO and HeLa cells, Chinese hamster ovary (CHO) cells, and WI38, BHK, and COS cell lines, although it will be appreciated by the skilled practitioner that other prokaryotic and eukaryotic cells and cell lines may be appropriate for a variety of purposes, e.g., to provide higher expression, post-translational modification, desirable glycosylation patterns, or other features.

Additionally, peptides, particularly shorter peptides, may be chemically synthesized, avoiding the need for purification from cells or culture media. It is known that peptides as short as 3 amino acids can act as an antigenic determinant and stimulate an immune response. Such peptides may be administered as vaccines in ISCOMs (Immune Stimulatory Complexes) as described by Janeway & Travers, Immunobiology: The Immune System In Health and Disease, 13.21 (Garland Publishing, Inc. New York, 1997). Accordingly, one aspect of the present invention includes small peptides encoded by the nucleic acid molecules disclosed herein. Such peptides include at least 5, and may be at least 10, 15, 20, 25, or 30 or more contiguous amino acids of the polypeptide sequences described herein.

EXAMPLE 3: Production of Antibodies Specific for Infection-Specific Antigens

Antibody against infection-specific antigen is encompassed by the present invention, particularly for the detection of *Chlamydia* infection-specific antigen. Such antibody may be produced by inoculation of an animal such as a guinea-pig or a monkey with infection-specific antigen produced as described above. Such antigen may be a polypeptide as disclosed herein, such as a complete or partial polypeptide from *C. psittaci*, *C. trachomatis*, *C. pneumoniae* or *C. pecorum*. As discussed above, any molecule that can elicit a specific, protective immune response

may be used as a vaccine, but since a minimum of three amino acids are required to do this, a vaccine should comprise at least three amino acids.

The peptide for use in the vaccine of the invention may be naturally derived or may be synthetic such as those synthesized on a commercially available peptide synthesizer. The peptide may also comprise a complete or partial peptide derived from the *C. pneumoniae* or *C. pecorum* infection-specific orthologs of the *C. trachomatis* or *C. psittaci* proteins as set out herein.

In one method of production, a polyclonal antibody is produced by providing a purified peptide which is diluted to 100 micrograms per milliliter in sterile saline and mixed with RiBi Trivalent Adjuvant (RiBi Immunochem Inc). The antigen/adjuvant emulsion is then administered to an anaesthetized guinea pig using a procedure as provided by the manufacturer. Serum is collected 14 days after secondary and tertiary immunizations.

Monoclonal antibody to epitopes of the *Chlamydia* peptides identified and isolated as described can be prepared from murine hybridomas according to the classical method of Kohler and Milstein (1975) or derivative methods thereof. Briefly, a mouse is repetitively inoculated with a few micrograms of the selected purified protein over a period of a few weeks. The mouse is then sacrificed, and the antibody-producing cells of the spleen isolated. The spleen cells are fused by means of polyethylene glycol with mouse myeloma cells, and the excess unfused cells destroyed by growth of the system on selective media comprising aminopterin, e.g., Hypoxanthene, Aminopterin and Thymidine (HAT) medium. The successfully fused cells are diluted and aliquots of the dilution placed in wells of a microtiter plate where growth of the culture is continued. Antibody-producing clones are identified by detection of antibody in the supernatant fluid of the wells by immunoassay procedures, such as ELISA, as originally described by Engvall (1980), and derivative methods thereof. Selected positive clones can be expanded and their monoclonal antibody product harvested for use. Detailed procedures for monoclonal antibody production are described in Harlow and Lane (1988).

An alternative approach to raising antibodies against the *Chlamydia* peptides is to use synthetic peptides synthesized on a commercially available peptide synthesizer based upon the amino acid sequence of the peptides predicted from nucleotide sequence data.

In another embodiment of the present invention, monoclonal antibodies that recognize a specific *Chlamydia* peptide are produced. Optimally, monoclonal antibodies will be specific to each peptide, i.e., such antibodies recognize and bind one *Chlamydia* peptide and do not substantially recognize or bind to other proteins, including those found in uninfected human cells.

The determination that an antibody specifically detects a particular *Chlamydia* peptide is made by any one of a number of standard immunoassay methods; for instance, the western blotting technique (Sambrook et al., 1989). To determine that a given antibody preparation (for instance from a guinea pig) specifically detects one *Chlamydia* peptide by western blotting, total cellular protein is extracted from a sample of blood from an unexposed subject and from a sample of blood from an exposed subject. As a positive control, total cellular protein is also extracted from

Chlamydia cells grown *in vitro*. These protein preparations are then electrophoresed on a sodium dodecyl sulfate-polyacrylamide gel. Thereafter, the proteins are transferred to a membrane (for example, nitrocellulose) by western blotting, and the antibody preparation is incubated with the membrane. After washing the membrane to remove non-specifically bound antibodies, the presence of specifically bound antibodies is detected by the use of an anti-guinea pig antibody conjugated to an enzyme such as alkaline phosphatase; application of the substrate 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium results in the production of a dense blue compound by immuno-localized alkaline phosphatase. Antibodies which specifically detect the *Chlamydia* protein will, by this technique, be shown to bind to the *Chlamydia*-extracted sample at a particular protein band (which will be localized at a given position on the gel determined by its molecular weight) and to the proteins extracted from the blood of the exposed subject. No significant binding will be detected to proteins from the unexposed subject.

EXAMPLE 4: Use of Infection-Specific Sequences and their Corresponding Peptides and Antibodies in Diagnostic Assays

Another aspect of the present invention is a method for detecting the presence of anti-*Chlamydia* antibodies that react with infection-specific *Chlamydia* proteins, *Chlamydia* peptides and *Chlamydia* nucleic acid sequences in biological samples. These methods include detection of antigen and antibody by ELISA and similar techniques, the detection of proteins in a tissue sample by immunofluorescence and related techniques and the detection of specific DNA sequences by specific hybridization and amplification.

One aspect of the invention is an ELISA that detects anti-*Chlamydia* antibodies in a medical specimen. An immunostimulatory infection-specific *Chlamydia* peptide of the present invention is employed as an antigen and is preferably bound to a solid matrix such as a crosslinked dextran such as SEPHADEX (Pharmacia, Piscataway, NJ), agarose, polystyrene, or the wells of a microtiter plate. The polypeptide is admixed with the specimen, such as blood, and the admixture is incubated for a sufficient time to allow antibodies present in the sample to immunoreact with the polypeptide. The presence of the positive immunoreaction is then determined using an ELISA assay, usually involving the use of an enzyme linked to an anti-immunoglobulin that catalyzes the conversion of a chromogenic substrate.

In one embodiment, the solid support to which the polypeptide is attached is the wall of a microtiter assay plate. After attachment of the polypeptide, any nonspecific binding sites on the microtiter well walls are blocked with a protein such as bovine serum albumin. Excess bovine serum albumin is removed by rinsing and the medical specimen is admixed with the polypeptide in the microtiter wells. After a sufficient incubation time, the microtiter wells are rinsed to remove excess sample and then a solution of a second antibody, capable of detecting human antibodies is added to the wells. This second antibody is typically linked to an enzyme such as peroxidase,

alkaline phosphatase or glucose oxidase. For example, the second antibody may be a peroxidase-labeled goat anti-human antibody. After further incubation, excess amounts of the second antibody are removed by rinsing and a solution containing a substrate for the enzyme label (such as hydrogen peroxide for the peroxidase enzyme) and a color-forming dye precursor, such as o-phenylenediamine is added. The combination of *Chlamydia* peptide (bound to the wall of the well), the human anti-*Chlamydia* antibodies (from the specimen), the enzyme-conjugated anti-human antibody and the color substrate will produce a color that can be read using an instrument that determines optical density, such as a spectrophotometer. These readings can be compared to a negative control such as a sample known to be free of anti-*Chlamydia* antibodies. Positive readings indicate the presence of anti-*Chlamydia* antibodies in the specimen, which in turn indicate a prior exposure of the patient to *Chlamydia*.

In another embodiment, antibodies that specifically recognize a *Chlamydia* peptide encoded by the nucleotide sequences disclosed herein are useful in diagnosing the presence of infection-specific *Chlamydia* antigens in a subject or sample. For example, detection of infection-specific antigens that are present in cells or tissues may be done by immunofluorescence, indirect-immunofluorescense and immunohistochemistry. In immunofluorescense, a fluorescent dye is bound directly to the antibody. In indirect-immunofluorescence, the dye is bound to an anti-immunoglobulin. Specific binding occurs between antigen and bound antibody is detected by virtue of fluorescent emissions from the dye moiety. This technique may be particularly useful, for instance, for detection of *Chlamydia* antigen present on a urogenital mucosal smear. *Chlamydia* may be present in urogenital mucosa, and a smear on a glass slide may be fixed and bathed in a solution containing an antibody specific to the infection-specific antigen. The slide is then washed to remove the unbound antibody, and a fluorescent anti-immunoglobulin antibody is added. The slide is washed again, and viewed microscopically under an appropriate wavelength of light to detect fluorescence. Fluorescence indicates the presence of *Chlamydia* antigen. Alternatively, a urogenital mucosal smear may be taken, the sample cultured with HeLa cells to produce large amounts of the RB form, and immunofluorescence may then be used to detect infection-specific *Chlamydia* antibodies.

Another aspect of the invention includes the use of nucleic acid primers to detect the presence of *Chlamydia* nucleic acids that encode infection-specific antigens in body samples and thus to diagnose infection. In other embodiments, these oligonucleotide primers will comprise at least 15 contiguous nucleotides of a DNA sequence as shown in SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, or 17. In other embodiments, such oligonucleotides may comprise at least 20 or at least 25 or more contiguous nucleotides of the aforementioned sequences.

One skilled in the art will appreciate that PCR primers are not required to exactly match the target gene sequence to which they anneal. Therefore, in another embodiment, the oligonucleotides will comprise a sequence of at least 15 nucleotides and preferably at least 20 nucleotides, the oligonucleotide sequence being substantially similar to a DNA sequence set forth

in SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, and 17. Such oligonucleotides may share at least about 75%, 85%, 90% or greater sequence identity.

The detection of specific nucleic acid sequences in a sample by polymerase chain reaction amplification (PCR) is discussed in detail in Innis et al., (1990). *PCR Protocols: A Guide to Methods and Applications*, Academic Press: San Diego, part 4 in particular. To detect *Chlamydia* sequences, primers based on the sequences disclosed herein would be synthesized, such that PCR amplification of a sample containing *Chlamydia* DNA would result in an amplified fragment of a predicted size. If necessary, the presence of this fragment following amplification of the sample nucleic acid could be detected by dot blot analysis. PCR amplification employing primers based on the sequences disclosed herein may also be employed to quantify the amounts of *Chlamydia* nucleic acid present in a particular sample (see chapters 8 and 9 of Innis et al., (1990)).

Alternatively, probes based on the nucleic acid sequences described herein may be labeled with suitable labels (such a P^{32} or biotin) and used in hybridization assays to detect the presence of *Chlamydia* nucleic acid in provided samples.

Reverse-transcription PCR using these primers may also be utilized to detect the presence of *Chlamydia* RNA which is indicative of an ongoing infection.

EXAMPLE 5: Production of Chlamydia Vaccines

The purified peptides of the present invention may be used directly as immunogens for vaccination. Methods for using purified peptides as vaccines are well known in the art and are described in Yang et al. (1991), Andersen (1994) and Jardim et al. (1990). As is well known in the art, adjuvants such as alum, Complete Freund's Adjuvant (CFA) and Incomplete Freund's Adjuvant (IFA) may be used in formulations of purified peptides as vaccines. Accordingly, one embodiment of the present invention is a vaccine comprising one or more immunostimulatory *C. trachomatis* or *C. psittaci* peptides encoded by nucleotide sequences as shown in the attached sequence listing, together with a pharmaceutically acceptable adjuvant.

Additionally a vaccine may comprise a defined fraction of the disclosed peptide of *C. trachomatis* or *C. psittaci* or may comprise a peptide wherein the gene coding for the peptide shows substantial similarity to the DNA sequences disclosed herein, such as for orthologous genes of *C. pneumoniae* or *C. pecorum*.

Additionally, the vaccines may be formulated using a peptide according to the present invention together with a pharmaceutically acceptable excipient such as water, saline, dextrose and glycerol. The vaccines may also include auxiliary substances such as emulsifying agents and pH buffers.

It will be appreciated by one of skill in the art that vaccines formulated as described above may be administered in a number of ways including subcutaneous, intra-muscular and intra-venous injection. Doses of the vaccine administered will vary depending on the antigenicity of the particular peptide or peptide combination employed in the vaccine, and characteristics of the

animal or human patient to be vaccinated. While the determination of individual doses will be within the skill of the administering physician, it is anticipated that doses of between 1 microgram and 1 milligram will be employed.

As with many vaccines, the vaccines of the present invention may routinely be administered several times over the course of a number of weeks to ensure that an effective immune response is triggered. Where such multiple doses are administered, they will normally be administered at from two to twelve week intervals, more usually from three to five week intervals. Periodic boosters at intervals of 1-5 years, usually three years, may be desirable to maintain the desired levels of protective immunity.

Alternatively, multiple immunostimulatory peptides may also be administered by expressing the nucleic acids encoding the peptides in a nonpathogenic microorganism, and using this transformed nonpathogenic microorganism as a vaccine.

Finally, a recent development in the field of vaccines is the direct injection of nucleic acid molecules encoding peptide antigens, as described in Janeway & Travers, (1997). Thus, plasmids which include nucleic acid molecules described herein, or which include nucleic acid sequences encoding peptides according to the present invention may be utilized in such DNA vaccination methods.

The vaccine of the invention may be used to inoculate potential animal targets of any of the chlamydial diseases including those caused by *C. trachomatis, C. psittaci, C. pneumoniae* or *C. pecorum*. Indeed the vaccine of the invention may be used to inoculate animals against any disease that shows immunological cross-protection as a result of exposure to infection-specific *Chlamydia* antigen. The protein or polypeptide is present in the vaccine in an amount sufficient to induce a protective immune response whether through humoral or cell mediated pathways or through both. Such a response protects the immunized animal against chlamydial infections specifically by raising an immune response against the Reticulate Body form of *Chlamydia*.

The above embodiments are set out only by way of example and are not intended to be exclusive, one skilled in the art will understand that the invention may be practiced in various additional ways without departing from the subject of the spirit of the invention.

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CLAIMS

What is claimed is:

1. A purified infection-specific protein comprising an amino acid sequence selected from the group consisting of:

(a) SEQ ID NO: 2,

(b) SEQ ID NO: 4,

(c) SEQ ID NO: 6,

(d) SEQ ID NO: 10,

(e) SEQ ID NO: 12,

(f) an amino acid sequence that differs from an amino acid sequence of (a) to (e) inclusive, by one or more conservative amino acid substitutions, and

(g) an amino acid sequence having at least 60% sequence identity to an amino acid sequence of (a) to (e) inclusive.

2. An isolated nucleic acid molecule encoding a protein according to claim 1.

3. An isolated nucleic acid molecule according to claim 2 wherein the nucleic acid molecule comprises a nucleic acid sequence selected from the group consisting of:

(a) SEQ ID NO: 1,

(b) SEQ ID NO: 3,

(c) SEQ ID NO: 5,

(d) SEQ ID NO: 9, and

(e) SEQ ID NO: 11.

4. A recombinant nucleic acid molecule comprising a promoter sequence operably linked to a nucleotide molecule according to claim 2.

5. A vaccine preparation comprising at least one purified peptide comprising at least 5 contiguous amino acids selected from the group consisting of:

(a) SEQ ID NO: 2,

(b) SEQ ID NO: 4,

(c) SEQ ID NO: 6,

(d) SEQ ID NO: 8,

(e) SEQ ID NO: 10,

(f) SEQ ID NO: 12,

(g) SEQ ID NO: 14,

(h) SEQ ID NO: 16, and

(i) SEQ ID NO: 18.

6. The vaccine preparation of claim 5 wherein the peptide comprises at least 10 contiguous amino acids of at least one of the specified sequences.

7. The vaccine preparation of claim 5 wherein the peptide comprises at least 15 contiguous amino acids of at least one of the specified sequences.

8. The vaccine preparation of claim 5 wherein the purified peptide comprises at least 20 contiguous amino acids of at least one of the specified sequences.

9. A vaccine preparation comprising an amino acid sequence selected from the group consisting of:

(a) SEQ ID NO: 2,

(b) SEQ ID NO: 4,

(c) SEQ ID NO: 6,

(d) SEQ ID NO: 8,

(e) SEQ ID NO: 10,

(f) SEQ ID NO: 12,

(g) SEQ ID NO: 14,

(h) SEQ ID NO: 16,

(i) SEQ ID NO: 18,

(j) an amino acid sequence that differs from an amino acid sequence of (a) to (i) inclusive, by one or more conservative amino acid substitutions, and

(k) an amino acid sequence having at least 60% sequence identity to an amino acid sequence of (a) to (i) inclusive.

10. A method of making a vaccine comprising combining a pharmaceutically acceptable excipient with a purified peptide having an amino acid sequence selected from the group consisting of:

(a) SEQ ID NO:2,

(b) SEQ ID NO:4,

(c) SEQ ID NO:6,

(d) SEQ ID NO:8,

(e) SEQ ID NO:10,

(f) SEQ ID NO:12,

(g) SEQ ID NO:14,

(h) SEQ ID NO:16,

(i) SEQ ID NO:18,

(j) an amino acid sequence that differs from an amino acid sequence of (a) to (i) inclusive, by one or more conservative amino acid substitutions,

(k) an amino acid sequence having at least 60% sequence identity to an amino acid sequence of (a) to (i) inclusive, and

(l) at least 10 contiguous amino acids from an amino acid sequence of (a) to (i) inclusive.

11. A method of vaccination, comprising administering a vaccine preparation according to claim 5 to a mammal.

12. A method of vaccination, comprising administering a vaccine preparation according to claim 9 to a mammal.

13. A method of detecting an infection-specific *Chlamydia* protein in a biological sample comprising: contacting the biological sample with at least one anti-*Chlamydia* antibody, which antibody is an infection-specific antibody, such that a reaction between the antibody and the infection-specific *Chlamydia* protein gives rise to a detectable effect, and detecting the detectable effect.

14. The method of claim 13 wherein the anti-*Chlamydia* antibody binds specifically to a peptide having an amino acid sequence selected from the group consisting of:

(a) SEQ ID NO: 2,

(b) SEQ ID NO: 4,

(c) SEQ ID NO: 6,

(d) SEQ ID NO: 8,

(e) SEQ ID NO: 10,

(f) SEQ ID NO: 12,

(g) SEQ ID NO: 14,

(h) SEQ ID NO: 16, and

(i) SEQ ID NO: 18.

15. A method of detecting an infection-specific anti-*Chlamydia* antibody in a biological sample comprising: contacting the biological sample with at least one *Chlamydia* peptide, which peptide is an infection specific peptide, such that a reaction between the peptide and the infection-specific anti-*Chlamydia* antibody gives rise to a detectable effect, and detecting the detectable effect.

16. The method of claim 15 wherein the *Chlamydia* peptide comprises at least 5 contiguous amino acids of a sequence selected from the group consisting of:

(a) SEQ ID NO: 2,

(b) SEQ ID NO: 4,

(c) SEQ ID NO: 6,

(d) SEQ ID NO: 8,

(e) SEQ ID NO: 10,

(f) SEQ ID NO: 12,

(g) SEQ ID NO: 14,

(h) SEQ ID NO: 16, and

(i) SEQ ID NO: 18.

17. The method of claim 15 wherein said *Chlamydia* peptide comprises an amino acid sequence selected from the group consisting of:

(a) SEQ ID NO: 2,

(b) SEQ ID NO: 4,

(c) SEQ ID NO: 6,

(d) SEQ ID NO: 8,

(e) SEQ ID NO: 10,

(f) SEQ ID NO: 12,

(g) SEQ ID NO: 14,

(h) SEQ ID NO: 16, and

(i) SEQ ID NO: 18.

18. A method of treating a *Chlamydial* infection comprising directing a therapeutic agent against a specific target, said target chosen from the group consisting of: (i) an infection-specific protein of *Chlamydia*, (ii) a gene that encodes an infection-specific protein of *Chlamydia* and (iii) an RNA transcript that encodes an infection-specific protein of *Chlamydia*, wherein said therapeutic agent interacts with said target to affect a reduction in pathology.

SEQUENCE LISTING

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                 85                  90                  95

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gct gtt att aaa gtt ctt gat gtt ctt ttc aaa ata att aac atg cga   528
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Arg Cys Leu Glu Glu Ser Ala Leu Gly Lys Lys Glu Ser Ala Glu Phe
        35                  40                  45

Glu Lys Met Lys Asn Gln Phe Ser Asn Ser Met Gly Lys Met Glu Glu
    50                  55                  60

Glu Leu Ser Ser Ile Tyr Ser Lys Leu Gln Asp Asp Asp Tyr Met Glu
65                  70                  75                  80

Gly Leu Ser Glu Thr Ala Ala Ala Glu Leu Arg Lys Lys Phe Glu Asp
                85                  90                  95

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            20                  25                  30

gag atg gtg aag ggg gat gaa gac cga atg gct atg agc cag ctg att   144
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         35                  40                  45

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Phe Cys Asn Gly Leu Gly Leu Glu His Ser Ala Ser Leu Arg Lys His
     50                  55                  60

cta gag ggt aac cca aaa gtc gtt gat tta ggt caa cgt ttg ctt aac   240
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 65                  70                  75                  80

aaa aac tgt ttt gat ctt ctg agt gaa gaa gga ttc cct gac cca cat   288
Lys Asn Cys Phe Asp Leu Leu Ser Glu Glu Gly Phe Pro Asp Pro His
                85                  90                  95

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Ile Trp Thr Asp Met Arg Val Trp Gly Ala Ala Val Lys Glu Met Ala
            100                 105                 110

gcg gca tta att caa caa ttt cct caa tat gaa gaa gat ttt caa aag   384
Ala Ala Leu Ile Gln Gln Phe Pro Gln Tyr Glu Glu Asp Phe Gln Lys
        115                 120                 125

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Asn Ala Asp Gln Ile Leu Ser Glu Met Glu Glu Leu Asp Arg Trp Ala
    130                 135                 140

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Val Arg Ser Leu Ser Thr Ile Pro Glu Lys Asn Arg Tyr Leu Val Thr
145                 150                 155                 160

ggc cac aat gcg ttc agt tac ttt act cgt cgg tat cta tcc tct gat   528
Gly His Asn Ala Phe Ser Tyr Phe Thr Arg Arg Tyr Leu Ser Ser Asp
                165                 170                 175

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 65                  70                  75                  80

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                 85                  90                  95

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            100                 105                 110

Ala Ala Leu Ile Gln Gln Phe Pro Gln Tyr Glu Glu Asp Phe Gln Lys
        115                 120                 125

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Met Ser Val Ile Thr Ile Leu Ala Arg Ser Ser Thr Met Phe Ala Gln
  1               5                  10                  15

tta cag aag aat tgg gag gga ctg ttc ttg aat aga gat aat gca att   96
Leu Gln Lys Asn Trp Glu Gly Leu Phe Leu Asn Arg Asp Asn Ala Ile
            20                  25                  30

gct tgg tcc gta gag gat ctt tgt gtt aat tat gat cac tca gac gtc   144
Ala Trp Ser Val Glu Asp Leu Cys Val Asn Tyr Asp His Ser Asp Val
        35                  40                  45

tta tgt cac att act ttt tct ctg cct gca ggg gca atg gct gct att   192
Leu Cys His Ile Thr Phe Ser Leu Pro Ala Gly Ala Met Ala Ala Ile
    50                  55                  60

att ggg ccg aat gga gct ggt aaa agt act ttg ctt aag gct tct tta   240
Ile Gly Pro Asn Gly Ala Gly Lys Ser Thr Leu Leu Lys Ala Ser Leu
65                  70                  75                  80

gga ctg att cgt gct tct tct ggc caa agc ttg ttc ttt ggt cag aga   288
Gly Leu Ile Arg Ala Ser Ser Gly Gln Ser Leu Phe Phe Gly Gln Arg
                85                  90                  95

ttt tcc aag gca cat cat aga ata gcc tat atg cct caa aga gcg agt   336
Phe Ser Lys Ala His His Arg Ile Ala Tyr Met Pro Gln Arg Ala Ser
```

```
            100                 105                 110

gtg gat tgg gat ttc cca atg act gtt ctt gat ctc gtg ttg atg ggg   384
Val Asp Trp Asp Phe Pro Met Thr Val Leu Asp Leu Val Leu Met Gly
        115                 120                 125

tgt tac ggc tat aaa gga ata tgg aat cgt att tcc act gat gat cgt   432
Cys Tyr Gly Tyr Lys Gly Ile Trp Asn Arg Ile Ser Thr Asp Asp Arg
    130                 135                 140

cag gag gct atg cgt att tta gag cgg gtt ggt ttg gaa gct ttt gca   480
Gln Glu Ala Met Arg Ile Leu Glu Arg Val Gly Leu Glu Ala Phe Ala
145                 150                 155                 160

aat cgt caa ata ggt aag ctc tct gga gga caa caa cag aga gct ttt   528
Asn Arg Gln Ile Gly Lys Leu Ser Gly Gly Gln Gln Gln Arg Ala Phe
                165                 170                 175

tta gcg cgg tca tta atg caa aaa gca gat ttg tat ctc atg gat gag   576
Leu Ala Arg Ser Leu Met Gln Lys Ala Asp Leu Tyr Leu Met Asp Glu
            180                 185                 190

ctg ttc tct gcg atc gat atg gcc tct tat cag atg gtt gta gat gtt   624
Leu Phe Ser Ala Ile Asp Met Ala Ser Tyr Gln Met Val Val Asp Val
        195                 200                 205

ttg caa gag ctt aaa agc gaa ggg aag act att gtg gtc att cat cat   672
Leu Gln Glu Leu Lys Ser Glu Gly Lys Thr Ile Val Val Ile His His
    210                 215                 220

gat ttg agt aat gtc cgg aag ctt ttt gat cat gtg att tta tta aat   720
Asp Leu Ser Asn Val Arg Lys Leu Phe Asp His Val Ile Leu Leu Asn
225                 230                 235                 240

aag cat ctt gtg tgc tct gga agc gta gaa gaa tgc ttg act aaa gaa   768
Lys His Leu Val Cys Ser Gly Ser Val Glu Glu Cys Leu Thr Lys Glu
                245                 250                 255

gcc att ttt cag gct tat ggg tgt gac ttg agc ttt tgg att aca cac   816
Ala Ile Phe Gln Ala Tyr Gly Cys Asp Leu Ser Phe Trp Ile Thr His
            260                 265                 270

tca aat tgt cta gag gca agt acc aag gat cgt gct aga tgc tga       861
Ser Asn Cys Leu Glu Ala Ser Thr Lys Asp Arg Ala Arg Cys
        275                 280                 285
```

<210> 6
<211> 286
<212> PRT
<213> Chlamydia trachomatis

<400> 6

```
Met Ser Val Ile Thr Ile Leu Ala Arg Ser Ser Thr Met Phe Ala Gln
  1               5                  10                  15

Leu Gln Lys Asn Trp Glu Gly Leu Phe Leu Asn Arg Asp Asn Ala Ile
            20                  25                  30

Ala Trp Ser Val Glu Asp Leu Cys Val Asn Tyr Asp His Ser Asp Val
        35                  40                  45
```

```
Leu Cys His Ile Thr Phe Ser Leu Pro Ala Gly Ala Met Ala Ala Ile
     50                  55                  60

Ile Gly Pro Asn Gly Ala Gly Lys Ser Thr Leu Leu Lys Ala Ser Leu
 65                  70                  75                  80

Gly Leu Ile Arg Ala Ser Ser Gly Gln Ser Leu Phe Phe Gly Gln Arg
                 85                  90                  95

Phe Ser Lys Ala His His Arg Ile Ala Tyr Met Pro Gln Arg Ala Ser
            100                 105                 110

Val Asp Trp Asp Phe Pro Met Thr Val Leu Asp Leu Val Leu Met Gly
        115                 120                 125

Cys Tyr Gly Tyr Lys Gly Ile Trp Asn Arg Ile Ser Thr Asp Asp Arg
    130                 135                 140

Gln Glu Ala Met Arg Ile Leu Glu Arg Val Gly Leu Glu Ala Phe Ala
145                 150                 155                 160

Asn Arg Gln Ile Gly Lys Leu Ser Gly Gly Gln Gln Gln Arg Ala Phe
                165                 170                 175

Leu Ala Arg Ser Leu Met Gln Lys Ala Asp Leu Tyr Leu Met Asp Glu
            180                 185                 190

Leu Phe Ser Ala Ile Asp Met Ala Ser Tyr Gln Met Val Val Asp Val
        195                 200                 205

Leu Gln Glu Leu Lys Ser Glu Gly Lys Thr Ile Val Val Ile His His
    210                 215                 220

Asp Leu Ser Asn Val Arg Lys Leu Phe Asp His Val Ile Leu Leu Asn
225                 230                 235                 240

Lys His Leu Val Cys Ser Gly Ser Val Glu Glu Cys Leu Thr Lys Glu
                245                 250                 255

Ala Ile Phe Gln Ala Tyr Gly Cys Asp Leu Ser Phe Trp Ile Thr His
            260                 265                 270

Ser Asn Cys Leu Glu Ala Ser Thr Lys Asp Arg Ala Arg Cys
        275                 280                 285
```

<210> 7
<211> 1068
<212> DNA
<213> Chlamydia psittaci

<220>
<221> CDS
<222> (1)..(1068)

<400> 7

```
atg aca gta tcc aca gac aac aca agt cct gta ata tcg aga gcg tcc   48
Met Thr Val Ser Thr Asp Asn Thr Ser Pro Val Ile Ser Arg Ala Ser
  1               5                  10                  15
```

```
tca cct act ttt gga gat cat ggt aag gat ttc gac aac aat aaa att   96
Ser Pro Thr Phe Gly Asp His Gly Lys Asp Phe Asp Asn Asn Lys Ile
             20                  25                  30

ata ccc att tca ata gaa gct cca act tct tca gct gct gct gta ggg   144
Ile Pro Ile Ser Ile Glu Ala Pro Thr Ser Ser Ala Ala Ala Val Gly
         35                  40                  45

gct aaa acg gct atc gag cct gaa gga aga agc cca cta ctt caa agg   192
Ala Lys Thr Ala Ile Glu Pro Glu Gly Arg Ser Pro Leu Leu Gln Arg
     50                  55                  60

att tgc tat ctt gtt aaa att atc gct gcc atc gcc ctc ttt gtt gtt   240
Ile Cys Tyr Leu Val Lys Ile Ile Ala Ala Ile Ala Leu Phe Val Val
 65                  70                  75                  80

ggt atc gca gcc tta gtt tgc tta tat ctc ggt agc gtt atc tca acg   288
Gly Ile Ala Ala Leu Val Cys Leu Tyr Leu Gly Ser Val Ile Ser Thr
                 85                  90                  95

cct tct ctt att ctt atg ctt gcg atc atg ctt gta tcc ttt gtg atc   336
Pro Ser Leu Ile Leu Met Leu Ala Ile Met Leu Val Ser Phe Val Ile
            100                 105                 110

gtt att acg gca att cga gat ggc aca ccg tct caa gtg gtc cgt cac   384
Val Ile Thr Ala Ile Arg Asp Gly Thr Pro Ser Gln Val Val Arg His
        115                 120                 125

atg aaa cag caa att cag caa ttt ggc gaa gaa aac acg cgt tta cat   432
Met Lys Gln Gln Ile Gln Gln Phe Gly Glu Glu Asn Thr Arg Leu His
    130                 135                 140

acc gca gta gaa aat cta aaa gct gtt aac gtt gag ctc tca gag caa   480
Thr Ala Val Glu Asn Leu Lys Ala Val Asn Val Glu Leu Ser Glu Gln
145                 150                 155                 160

att aac caa ctt aaa caa cta cat act aga tta tcg gat ttt ggt gat   528
Ile Asn Gln Leu Lys Gln Leu His Thr Arg Leu Ser Asp Phe Gly Asp
                165                 170                 175

agg ctt gaa gcg aat acc ggt gat ttt act gca ctt att gcg gat ttc   576
Arg Leu Glu Ala Asn Thr Gly Asp Phe Thr Ala Leu Ile Ala Asp Phe
            180                 185                 190

caa ctc agt ctg gaa gag ttt aag tct gtt ggt act aaa gtt gaa acc   624
Gln Leu Ser Leu Glu Glu Phe Lys Ser Val Gly Thr Lys Val Glu Thr
        195                 200                 205

atg ctc tct cca ttt gag aaa tta gct cag tct ttg aaa gag acc ttt   672
Met Leu Ser Pro Phe Glu Lys Leu Ala Gln Ser Leu Lys Glu Thr Phe
    210                 215                 220

tct caa gaa gct gtt cag gca atg atg tcc tct gta act gag tta aga   720
Ser Gln Glu Ala Val Gln Ala Met Met Ser Ser Val Thr Glu Leu Arg
225                 230                 235                 240

acc aat ttg aat gca ttg aaa gag ctt ata aca gag aat aaa acc gta   768
Thr Asn Leu Asn Ala Leu Lys Glu Leu Ile Thr Glu Asn Lys Thr Val
                245                 250                 255

ata gag caa cta aaa gct gat gct caa ctt aga gaa gag caa gtg cgg   816
```

```
Ile Glu Gln Leu Lys Ala Asp Ala Gln Leu Arg Glu Glu Gln Val Arg
            260                 265                 270

ttt tta gaa aag cgt aaa caa gag tta gaa gag gct tgt tca aca ttg   864
Phe Leu Glu Lys Arg Lys Gln Glu Leu Glu Glu Ala Cys Ser Thr Leu
        275                 280                 285

tcc cat tca att gcg act cta cag gaa tcc aca acc ctt cta aag gac   912
Ser His Ser Ile Ala Thr Leu Gln Glu Ser Thr Thr Leu Leu Lys Asp
    290                 295                 300

tct aca act aac tta cat gca gtt gaa agt cgt ctt atc ggt gtt atg   960
Ser Thr Thr Asn Leu His Ala Val Glu Ser Arg Leu Ile Gly Val Met
305                 310                 315                 320

gtt cag gat ggt gca gag tcc tcc acc gta gag gaa gct tca caa gat  1008
Val Gln Asp Gly Ala Glu Ser Ser Thr Val Glu Glu Ala Ser Gln Asp
                325                 330                 335

gat agc gcg caa ccc caa gat gaa aat caa tct gat gct gga gag cat  1056
Asp Ser Ala Gln Pro Gln Asp Glu Asn Gln Ser Asp Ala Gly Glu His
            340                 345                 350

aaa gat agt taa                                                 1068
Lys Asp Ser
        355
```

<210> 8
<211> 355
<212> PRT
<213> Chlamydia psittaci

<400> 8

```
Met Thr Val Ser Thr Asp Asn Thr Ser Pro Val Ile Ser Arg Ala Ser
  1               5                  10                  15

Ser Pro Thr Phe Gly Asp His Gly Lys Asp Phe Asp Asn Asn Lys Ile
             20                  25                  30

Ile Pro Ile Ser Ile Glu Ala Pro Thr Ser Ser Ala Ala Ala Val Gly
         35                  40                  45

Ala Lys Thr Ala Ile Glu Pro Glu Gly Arg Ser Pro Leu Leu Gln Arg
     50                  55                  60

Ile Cys Tyr Leu Val Lys Ile Ile Ala Ala Ile Ala Leu Phe Val Val
 65                  70                  75                  80

Gly Ile Ala Ala Leu Val Cys Leu Tyr Leu Gly Ser Val Ile Ser Thr
                 85                  90                  95

Pro Ser Leu Ile Leu Met Leu Ala Ile Met Leu Val Ser Phe Val Ile
            100                 105                 110

Val Ile Thr Ala Ile Arg Asp Gly Thr Pro Ser Gln Val Val Arg His
        115                 120                 125

Met Lys Gln Gln Ile Gln Gln Phe Gly Glu Glu Asn Thr Arg Leu His
    130                 135                 140
```

```
Thr Ala Val Glu Asn Leu Lys Ala Val Asn Val Glu Leu Ser Glu Gln
145                 150                 155                 160

Ile Asn Gln Leu Lys Gln Leu His Thr Arg Leu Ser Asp Phe Gly Asp
                165                 170                 175

Arg Leu Glu Ala Asn Thr Gly Asp Phe Thr Ala Leu Ile Ala Asp Phe
            180                 185                 190

Gln Leu Ser Leu Glu Glu Phe Lys Ser Val Gly Thr Lys Val Glu Thr
        195                 200                 205

Met Leu Ser Pro Phe Glu Lys Leu Ala Gln Ser Leu Lys Glu Thr Phe
    210                 215                 220

Ser Gln Glu Ala Val Gln Ala Met Met Ser Ser Val Thr Glu Leu Arg
225                 230                 235                 240

Thr Asn Leu Asn Ala Leu Lys Glu Leu Ile Thr Glu Asn Lys Thr Val
                245                 250                 255

Ile Glu Gln Leu Lys Ala Asp Ala Gln Leu Arg Glu Glu Gln Val Arg
            260                 265                 270

Phe Leu Glu Lys Arg Lys Gln Glu Leu Glu Glu Ala Cys Ser Thr Leu
        275                 280                 285

Ser His Ser Ile Ala Thr Leu Gln Glu Ser Thr Thr Leu Leu Lys Asp
    290                 295                 300

Ser Thr Thr Asn Leu His Ala Val Glu Ser Arg Leu Ile Gly Val Met
305                 310                 315                 320

Val Gln Asp Gly Ala Glu Ser Ser Thr Val Glu Glu Ala Ser Gln Asp
                325                 330                 335

Asp Ser Ala Gln Pro Gln Asp Glu Asn Gln Ser Asp Ala Gly Glu His
            340                 345                 350

Lys Asp Ser
        355
```

<210> 9
<211> 597
<212> DNA
<213> Chlamydia psittaci

<220>
<221> CDS
<222> (1)..(597)

<400> 9

```
atg tca aca aca cca gca tct tca gca agt cga gac gta tta tta gat   48
Met Ser Thr Thr Pro Ala Ser Ser Ala Ser Arg Asp Val Leu Leu Asp
  1               5                  10                  15

gac gtt tta ata gct ttt aat aga aag cta aat ctc gta gaa caa caa   96
Asp Val Leu Ile Ala Phe Asn Arg Lys Leu Asn Leu Val Glu Gln Gln
             20                  25                  30
```

```
gcg aaa gaa ctt gaa acg aaa gtc agt ttg gta gac aga aca gct act   144
Ala Lys Glu Leu Glu Thr Lys Val Ser Leu Val Asp Arg Thr Ala Thr
         35                  40                  45

tta tca ctt acc act ggc aat aat gta gcc acg gat gta ctc ctt tta   192
Leu Ser Leu Thr Thr Gly Asn Asn Val Ala Thr Asp Val Leu Leu Leu
     50                  55                  60

aaa gat gag gtt gca gaa cta aaa gga tgt ttg tct gca gtt acg gat   240
Lys Asp Glu Val Ala Glu Leu Lys Gly Cys Leu Ser Ala Val Thr Asp
 65                  70                  75                  80

cta tta atc cgc tca ggc tca tca aga aca cct ggg ggt gct cct aat   288
Leu Leu Ile Arg Ser Gly Ser Ser Arg Thr Pro Gly Gly Ala Pro Asn
                 85                  90                  95

cca gaa ggc act aat tac cta ata gga tgc aca cct cct tct ctt tgc   336
Pro Glu Gly Thr Asn Tyr Leu Ile Gly Cys Thr Pro Pro Ser Leu Cys
            100                 105                 110

gct aaa ctt aca gcg tta gcg tta aca att ata gcc ctc att gct atc   384
Ala Lys Leu Thr Ala Leu Ala Leu Thr Ile Ile Ala Leu Ile Ala Ile
        115                 120                 125

aca gta ctt gtt atc tgt att gtt act gtt tgc ggc ggt ttc ccc cta   432
Thr Val Leu Val Ile Cys Ile Val Thr Val Cys Gly Gly Phe Pro Leu
    130                 135                 140

ttt att tcc cta ctc aac atg tac aca gtt ggt gct tgt ata tcc tta   480
Phe Ile Ser Leu Leu Asn Met Tyr Thr Val Gly Ala Cys Ile Ser Leu
145                 150                 155                 160

ccg atc att tcg tgt gcc gca gtt tca atg atg att cta tgc tca cat   528
Pro Ile Ile Ser Cys Ala Ala Val Ser Met Met Ile Leu Cys Ser His
                165                 170                 175

tct att aac tct tta tta aga aac agg cct gcg atc tat atg act aac   576
Ser Ile Asn Ser Leu Leu Arg Asn Arg Pro Ala Ile Tyr Met Thr Asn
            180                 185                 190

aat ttt caa aca gaa tct taa                                      597
Asn Phe Gln Thr Glu Ser
        195
```

<210> 10
<211> 198
<212> PRT
<213> Chlamydia psittaci

<400> 10

```
Met Ser Thr Thr Pro Ala Ser Ser Ala Ser Arg Asp Val Leu Leu Asp
  1               5                  10                  15

Asp Val Leu Ile Ala Phe Asn Arg Lys Leu Asn Leu Val Glu Gln Gln
             20                  25                  30

Ala Lys Glu Leu Glu Thr Lys Val Ser Leu Val Asp Arg Thr Ala Thr
         35                  40                  45

Leu Ser Leu Thr Thr Gly Asn Asn Val Ala Thr Asp Val Leu Leu Leu
```

```
     50                  55                  60

Lys Asp Glu Val Ala Glu Leu Lys Gly Cys Leu Ser Ala Val Thr Asp
 65                  70                  75                  80

Leu Leu Ile Arg Ser Gly Ser Ser Arg Thr Pro Gly Gly Ala Pro Asn
                 85                  90                  95

Pro Glu Gly Thr Asn Tyr Leu Ile Gly Cys Thr Pro Pro Ser Leu Cys
            100                 105                 110

Ala Lys Leu Thr Ala Leu Ala Leu Thr Ile Ile Ala Leu Ile Ala Ile
        115                 120                 125

Thr Val Leu Val Ile Cys Ile Val Thr Val Cys Gly Gly Phe Pro Leu
    130                 135                 140

Phe Ile Ser Leu Leu Asn Met Tyr Thr Val Gly Ala Cys Ile Ser Leu
145                 150                 155                 160

Pro Ile Ile Ser Cys Ala Ala Val Ser Met Met Ile Leu Cys Ser His
                165                 170                 175

Ser Ile Asn Ser Leu Leu Arg Asn Arg Pro Ala Ile Tyr Met Thr Asn
            180                 185                 190

Asn Phe Gln Thr Glu Ser
        195
```

<210> 11
<211> 561
<212> DNA
<213> Chlamydia psittaci

<220>
<221> CDS
<222> (1)..(561)

<400> 11

```
atg acc tct gta aga acc gat tta act cca ggc gac acc tca ctc caa   48
Met Thr Ser Val Arg Thr Asp Leu Thr Pro Gly Asp Thr Ser Leu Gln
  1               5                  10                  15

tct tct tta tta aat ccg agt gat ctc aca aca caa cta tcc aac ctc   96
Ser Ser Leu Leu Asn Pro Ser Asp Leu Thr Thr Gln Leu Ser Asn Leu
             20                  25                  30

cag act gtt ctc gca ggg ata caa caa caa cat cct tta aac ggt ggt   144
Gln Thr Val Leu Ala Gly Ile Gln Gln Gln His Pro Leu Asn Gly Gly
         35                  40                  45

tgg cct cag cat cat cct act ggc gct gca gat caa aat tat ctc atg   192
Trp Pro Gln His His Pro Thr Gly Ala Ala Asp Gln Asn Tyr Leu Met
     50                  55                  60

cgt ctg atg caa tct cat atg gca agt acc gta tca gca gta tct gaa   240
Arg Leu Met Gln Ser His Met Ala Ser Thr Val Ser Ala Val Ser Glu
 65                  70                  75                  80

tta aga acc gaa gtc act gca atc aag aca aaa ttg cac ggg cta tct   288
```

```
Leu Arg Thr Glu Val Thr Ala Ile Lys Thr Lys Leu His Gly Leu Ser
                 85                  90                  95

act cca gct aat gtt tgc agc ggt cct atg gct cta gcc gct ttt ctt   336
Thr Pro Ala Asn Val Cys Ser Gly Pro Met Ala Leu Ala Ala Phe Leu
            100                 105                 110

cta gct ata tct tta gtt gcg att atc atc att gtt tta gcc tcc tta   384
Leu Ala Ile Ser Leu Val Ala Ile Ile Ile Ile Val Leu Ala Ser Leu
        115                 120                 125

ggc ctt gca ggc ata cta cct caa gct gcc gct atc tta gtg aat aca   432
Gly Leu Ala Gly Ile Leu Pro Gln Ala Ala Ala Ile Leu Val Asn Thr
    130                 135                 140

gca aac tct ata tgg gct att gtt agc gct tcg ata gtc act gtt atc   480
Ala Asn Ser Ile Trp Ala Ile Val Ser Ala Ser Ile Val Thr Val Ile
145                 150                 155                 160

tgc tta att agc gtg cta tgc ata acg cta att cga cac cat aaa ccc   528
Cys Leu Ile Ser Val Leu Cys Ile Thr Leu Ile Arg His His Lys Pro
                165                 170                 175

tta cct att gaa act agg cct acc gga cat taa                      561
Leu Pro Ile Glu Thr Arg Pro Thr Gly His
            180                 185
```

<210> 12
<211> 186
<212> PRT
<213> Chlamydia psittaci

<400> 12

```
Met Thr Ser Val Arg Thr Asp Leu Thr Pro Gly Asp Thr Ser Leu Gln
  1               5                  10                  15

Ser Ser Leu Leu Asn Pro Ser Asp Leu Thr Thr Gln Leu Ser Asn Leu
             20                  25                  30

Gln Thr Val Leu Ala Gly Ile Gln Gln Gln His Pro Leu Asn Gly Gly
         35                  40                  45

Trp Pro Gln His His Pro Thr Gly Ala Ala Asp Gln Asn Tyr Leu Met
     50                  55                  60

Arg Leu Met Gln Ser His Met Ala Ser Thr Val Ser Ala Val Ser Glu
 65                  70                  75                  80

Leu Arg Thr Glu Val Thr Ala Ile Lys Thr Lys Leu His Gly Leu Ser
                 85                  90                  95

Thr Pro Ala Asn Val Cys Ser Gly Pro Met Ala Leu Ala Ala Phe Leu
            100                 105                 110

Leu Ala Ile Ser Leu Val Ala Ile Ile Ile Ile Val Leu Ala Ser Leu
        115                 120                 125

Gly Leu Ala Gly Ile Leu Pro Gln Ala Ala Ala Ile Leu Val Asn Thr
    130                 135                 140
```

```
Ala Asn Ser Ile Trp Ala Ile Val Ser Ala Ser Ile Val Thr Val Ile
145                 150                 155                 160

Cys Leu Ile Ser Val Leu Cys Ile Thr Leu Ile Arg His His Lys Pro
                165                 170                 175

Leu Pro Ile Glu Thr Arg Pro Thr Gly His
            180                 185
```

<210> 13
<211> 822
<212> DNA
<213> Chlamydia trachomatis

<220>
<221> CDS
<222> (1)..(822)

<400> 13

```
atg aca acg cct act cta atc gtg att cct cca tct ccc cct gca cct   48
Met Thr Thr Pro Thr Leu Ile Val Ile Pro Pro Ser Pro Pro Ala Pro
  1               5                  10                  15

tcc tac tca gcc aat cgc gta cct caa cct tct ttg atg gac aaa att   96
Ser Tyr Ser Ala Asn Arg Val Pro Gln Pro Ser Leu Met Asp Lys Ile
             20                  25                  30

aag aaa ata gca gcc att gcc tcc cta att ctt ata ggc aca ata ggc   144
Lys Lys Ile Ala Ala Ile Ala Ser Leu Ile Leu Ile Gly Thr Ile Gly
         35                  40                  45

ttt tta gct ctt ttg gga cat ctt gtt ggc ttt ctg atc gct cca caa   192
Phe Leu Ala Leu Leu Gly His Leu Val Gly Phe Leu Ile Ala Pro Gln
     50                  55                  60

atc act att gtt ctt ctt gcc cta ttc att acc tca tta gca ggg aat   240
Ile Thr Ile Val Leu Leu Ala Leu Phe Ile Thr Ser Leu Ala Gly Asn
 65                  70                  75                  80

gct ctt tat cta cag aaa acc gct aat cta cat cta tac cag gat ctg   288
Ala Leu Tyr Leu Gln Lys Thr Ala Asn Leu His Leu Tyr Gln Asp Leu
                85                  90                  95

caa aga gaa gtt ggg tct cta aaa gaa att aat ttc atg ctg agc gtt   336
Gln Arg Glu Val Gly Ser Leu Lys Glu Ile Asn Phe Met Leu Ser Val
            100                 105                 110

cta cag aaa gaa ttt ctt cat tta tct aaa gaa ttt gca acg aca tct   384
Leu Gln Lys Glu Phe Leu His Leu Ser Lys Glu Phe Ala Thr Thr Ser
        115                 120                 125

aaa gac ctc tct gct gta tct caa gat ttt tat tct tgt ttg caa gga   432
Lys Asp Leu Ser Ala Val Ser Gln Asp Phe Tyr Ser Cys Leu Gln Gly
    130                 135                 140

ttt aga gat aac tat aaa ggt ttt gaa tct ctt ttg gat gag tat aaa   480
Phe Arg Asp Asn Tyr Lys Gly Phe Glu Ser Leu Leu Asp Glu Tyr Lys
145                 150                 155                 160

aac tct aca gaa gaa atg cgc aaa ctc ttt tcg caa gaa atc ata gca   528
```

```
Asn Ser Thr Glu Glu Met Arg Lys Leu Phe Ser Gln Glu Ile Ile Ala
                165                 170                 175

gat ctt aaa ggc tct gtt gcc tca tta aga gag gaa atc cga ttc cta   576
Asp Leu Lys Gly Ser Val Ala Ser Leu Arg Glu Glu Ile Arg Phe Leu
            180                 185                 190

acc cca tta gca gaa gaa gtt cgc cga tta gcg cat aac cag gaa tca   624
Thr Pro Leu Ala Glu Glu Val Arg Arg Leu Ala His Asn Gln Glu Ser
        195                 200                 205

tta aca gcg gct att gaa gaa tta aaa aca att cgt gat agc tta cga   672
Leu Thr Ala Ala Ile Glu Glu Leu Lys Thr Ile Arg Asp Ser Leu Arg
    210                 215                 220

gat gaa att gga caa ctt tca caa ctt tct aaa act ctt acc agt caa   720
Asp Glu Ile Gly Gln Leu Ser Gln Leu Ser Lys Thr Leu Thr Ser Gln
225                 230                 235                 240

att gca tta caa cga aaa gag agc tca gat ctg tgt tcc cag ata aga   768
Ile Ala Leu Gln Arg Lys Glu Ser Ser Asp Leu Cys Ser Gln Ile Arg
                245                 250                 255

gag acg ctc tcc tcc ccc aga aag tct gca tca ccc tct aca aaa agc   816
Glu Thr Leu Ser Ser Pro Arg Lys Ser Ala Ser Pro Ser Thr Lys Ser
            260                 265                 270

tcc tag                                                          822
Ser
```

<210> 14
<211> 273
<212> PRT
<213> Chlamydia trachomatis

<400> 14

```
Met Thr Thr Pro Thr Leu Ile Val Ile Pro Pro Ser Pro Pro Ala Pro
  1               5                  10                  15

Ser Tyr Ser Ala Asn Arg Val Pro Gln Pro Ser Leu Met Asp Lys Ile
             20                  25                  30

Lys Lys Ile Ala Ala Ile Ala Ser Leu Ile Leu Ile Gly Thr Ile Gly
         35                  40                  45

Phe Leu Ala Leu Leu Gly His Leu Val Gly Phe Leu Ile Ala Pro Gln
     50                  55                  60

Ile Thr Ile Val Leu Leu Ala Leu Phe Ile Thr Ser Leu Ala Gly Asn
 65                  70                  75                  80

Ala Leu Tyr Leu Gln Lys Thr Ala Asn Leu His Leu Tyr Gln Asp Leu
                 85                  90                  95

Gln Arg Glu Val Gly Ser Leu Lys Glu Ile Asn Phe Met Leu Ser Val
            100                 105                 110

Leu Gln Lys Glu Phe Leu His Leu Ser Lys Glu Phe Ala Thr Thr Ser
        115                 120                 125
```

```
Lys Asp Leu Ser Ala Val Ser Gln Asp Phe Tyr Ser Cys Leu Gln Gly
    130                 135                 140

Phe Arg Asp Asn Tyr Lys Gly Phe Glu Ser Leu Leu Asp Glu Tyr Lys
145                 150                 155                 160

Asn Ser Thr Glu Glu Met Arg Lys Leu Phe Ser Gln Glu Ile Ile Ala
                165                 170                 175

Asp Leu Lys Gly Ser Val Ala Ser Leu Arg Glu Glu Ile Arg Phe Leu
            180                 185                 190

Thr Pro Leu Ala Glu Glu Val Arg Arg Leu Ala His Asn Gln Glu Ser
        195                 200                 205

Leu Thr Ala Ala Ile Glu Glu Leu Lys Thr Ile Arg Asp Ser Leu Arg
    210                 215                 220

Asp Glu Ile Gly Gln Leu Ser Gln Leu Ser Lys Thr Leu Thr Ser Gln
225                 230                 235                 240

Ile Ala Leu Gln Arg Lys Glu Ser Ser Asp Leu Cys Ser Gln Ile Arg
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Glu Thr Leu Ser Ser Pro Arg Lys Ser Ala Ser Pro Ser Thr Lys Ser
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Ser
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<210> 15
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<220>
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<222> (1)..(348)

<400> 15

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Met Val His Ser Val Tyr Asn Ser Leu Ala Pro Glu Gly Phe Ser Gln
  1               5                  10                  15

gtc tct att caa ccc agt cag att cca acc agc aaa aaa gta atg att   96
Val Ser Ile Gln Pro Ser Gln Ile Pro Thr Ser Lys Lys Val Met Ile
             20                  25                  30

gcg ata atg act ctt ttt gca ctc aca gcc att gca gca ata gtc ctt   144
Ala Ile Met Thr Leu Phe Ala Leu Thr Ala Ile Ala Ala Ile Val Leu
         35                  40                  45

tcc atc gtt aca gtt tgt gga ggg ttt cct ttt ctt ctt gct gca ctt   192
Ser Ile Val Thr Val Cys Gly Gly Phe Pro Phe Leu Leu Ala Ala Leu
     50                  55                  60

aac acc gta act att ggt gca tgc gta tcc ttg ccg gta ttc act tgc   240
Asn Thr Val Thr Ile Gly Ala Cys Val Ser Leu Pro Val Phe Thr Cys
 65                  70                  75                  80

ata gct aca acg tta tta ctt ctt tgt ctc cgt aat atc gaa ctc cta   288
```

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Ile Ala Thr Thr Leu Leu Leu Leu Cys Leu Arg Asn Ile Glu Leu Leu
                85                  90                  95

gcc aga ccg caa gta ttt acc ctc tcc act caa ttc agc cca aca aaa   336
Ala Arg Pro Gln Val Phe Thr Leu Ser Thr Gln Phe Ser Pro Thr Lys
            100                 105                 110

cct caa gaa tag                                                   348
Pro Gln Glu
        115
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<210> 16
<211> 115
<212> PRT
<213> Chlamydia trachomatis

<400> 16

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Met Val His Ser Val Tyr Asn Ser Leu Ala Pro Glu Gly Phe Ser Gln
  1               5                  10                  15

Val Ser Ile Gln Pro Ser Gln Ile Pro Thr Ser Lys Lys Val Met Ile
             20                  25                  30

Ala Ile Met Thr Leu Phe Ala Leu Thr Ala Ile Ala Ala Ile Val Leu
         35                  40                  45

Ser Ile Val Thr Val Cys Gly Gly Phe Pro Phe Leu Leu Ala Ala Leu
     50                  55                  60

Asn Thr Val Thr Ile Gly Ala Cys Val Ser Leu Pro Val Phe Thr Cys
 65                  70                  75                  80

Ile Ala Thr Thr Leu Leu Leu Leu Cys Leu Arg Asn Ile Glu Leu Leu
                85                  90                  95

Ala Arg Pro Gln Val Phe Thr Leu Ser Thr Gln Phe Ser Pro Thr Lys
            100                 105                 110

Pro Gln Glu
        115
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<210> 17
<211> 537
<212> DNA
<213> Chlamydia trachomatis

<220>
<221> CDS
<222> (1)..(537)

<400> 17

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Met Thr Tyr Ser Ile Ser Asp Ile Ala His Lys Ser Asp Ile Ser Asn
  1               5                  10                  15

ccc acg tct ccc gct cca tca aga aaa cga gga tcc ttt ccc cca caa   96
Pro Thr Ser Pro Ala Pro Ser Arg Lys Arg Gly Ser Phe Pro Pro Gln
             20                  25                  30
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```
tct cct tct gcc gtg ggc tct tta gag gga gct aat ttc tct aca tgg   144
Ser Pro Ser Ala Val Gly Ser Leu Glu Gly Ala Asn Phe Ser Thr Trp
         35                  40                  45

ggg cca ggc ccc ttc ttc act gtc cct gtt tat cca caa caa ctc gct   192
Gly Pro Gly Pro Phe Phe Thr Val Pro Val Tyr Pro Gln Gln Leu Ala
     50                  55                  60

gca atg caa aac aac ctt ttt aca ttg caa aca gag gtt tct gct ctc   240
Ala Met Gln Asn Asn Leu Phe Thr Leu Gln Thr Glu Val Ser Ala Leu
 65                  70                  75                  80

aag aaa aaa tta gtt cag tct agt cag aca cgc gga tct tta gga ctc   288
Lys Lys Lys Leu Val Gln Ser Ser Gln Thr Arg Gly Ser Leu Gly Leu
                 85                  90                  95

ggc ccg cag ttt tta gcg gca tgc tta gtt gct gcg aca atc ctt gca   336
Gly Pro Gln Phe Leu Ala Ala Cys Leu Val Ala Ala Thr Ile Leu Ala
            100                 105                 110

gta gct gtt atc gta ctt gct tcc tta gga ctt ggc ggt gtt ctt cct   384
Val Ala Val Ile Val Leu Ala Ser Leu Gly Leu Gly Gly Val Leu Pro
        115                 120                 125

ttt gtc ctt gtt tgt ctg gct ggg tca act aat gca att tgg gct att   432
Phe Val Leu Val Cys Leu Ala Gly Ser Thr Asn Ala Ile Trp Ala Ile
    130                 135                 140

gtg agc gcc tcc atc act aca ctg att tgt tgc gtt tcc atc gct tgc   480
Val Ser Ala Ser Ile Thr Thr Leu Ile Cys Cys Val Ser Ile Ala Cys
145                 150                 155                 160

atc ttc tta gca aaa tgt gat aag gga tct gat cct caa act tta tat   528
Ile Phe Leu Ala Lys Cys Asp Lys Gly Ser Asp Pro Gln Thr Leu Tyr
                165                 170                 175

gta agc taa                                                      537
Val Ser
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<210> 18
<211> 178
<212> PRT
<213> Chlamydia trachomatis

<400> 18

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  1               5                  10                  15

Pro Thr Ser Pro Ala Pro Ser Arg Lys Arg Gly Ser Phe Pro Pro Gln
             20                  25                  30

Ser Pro Ser Ala Val Gly Ser Leu Glu Gly Ala Asn Phe Ser Thr Trp
         35                  40                  45

Gly Pro Gly Pro Phe Phe Thr Val Pro Val Tyr Pro Gln Gln Leu Ala
     50                  55                  60

Ala Met Gln Asn Asn Leu Phe Thr Leu Gln Thr Glu Val Ser Ala Leu
 65                  70                  75                  80
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Lys Lys Lys Leu Val Gln Ser Ser Gln Thr Arg Gly Ser Leu Gly Leu
85 90 95

Gly Pro Gln Phe Leu Ala Ala Cys Leu Val Ala Ala Thr Ile Leu Ala
100 105 110

Val Ala Val Ile Val Leu Ala Ser Leu Gly Leu Gly Gly Val Leu Pro
115 120 125

Phe Val Leu Val Cys Leu Ala Gly Ser Thr Asn Ala Ile Trp Ala Ile
130 135 140

Val Ser Ala Ser Ile Thr Thr Leu Ile Cys Cys Val Ser Ile Ala Cys
145 150 155 160

Ile Phe Leu Ala Lys Cys Asp Lys Gly Ser Asp Pro Gln Thr Leu Tyr
165 170 175

Val Ser

<210> 19
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: PCR primer

<400> 19
agaaccgatt taactccagg cg 22

<210> 20
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: PCR primer

<400> 20
gcgcggatcc ttaatgtccg gtaggcctag 30

<210> 21
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: PCR primer

<400> 21
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<210> 22
<211> 34
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: PCR primer

<400> 22
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<210> 23
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<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: PCR primer

<400> 23
cgcagtactg tatccacaga caac 24

<210> 24
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: PCR primer

<400> 24
gtcggatccg agaaactctc catgcc 26

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/08744

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 39/00, 39/118, 49/00; G01N 33/571

US CL :424/9.2, 184.1, 263.1; 435/7.36

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/9.2, 184.1, 263.1; 435/7.36

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DYER et al. Analysis of a cation-transporting ATPase of Plasmodium falciparum. Molecular and Biochemical Parasitology. March 1996, Vol. 78, pages 1-12, especially Figure 1.	5, 6
X,P	STEPHENS et al. Genome sequence of an obligate intracellular pathogen of humans: Chlamydia trachomatis. Science. October 1998, Vol. 282, No. 5389, pages 754-759, especially page 754, column 3.	2, 3, 4

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

20 JULY 1999

Date of mailing of the international search report

23 AUG 1999

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

RODNEY P. SWARTZ, PH.D.

Telephone No. (703)308-0196

Form PCT/ISA/210 (second sheet)(July 1992)★

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/08744

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-12 drawn to p242 protein

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/08744

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, BIOSIS, CABA, CAPLUS, EMBASE, EMBAL, GENBANK, LIFESCI, MEDLINE, SCISEARCH
search terms: chlamydia, trachomatis, sequence id numbers, vaccine, reticulate body, elementary body, p242 protein

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claims 1-12, drawn to p242 *C. tracomatis* protein (SEQ ID NO:2), DNA (SEQ ID NO:1), method of making, and first method of use (vaccination).
Group II, claims 1-12, drawn to TroA *C. tracomatis* protein (SEQ ID NO:4), DNA (SEQ ID NO:3), method of making, and first method of use (vaccination).
Group III, claims 1-12, drawn to TroB *C. tracomatis* protein (SEQ ID NO:6), DNA (SEQ ID NO:5), method of making, and first method of use (vaccination).
Group IV, claims 1-12, drawn to IncB *C. psittaci* protein (SEQ ID NO:10), DNA (SEQ ID NO:9), method of making, and first method of use (vaccination).
Group V, claims 1-12, drawn to IncC *C. psittaci* protein (SEQ ID NO:12), DNA (SEQ ID NO:11), method of making, and first method of use (vaccination).
Group VI, claims 5-12, drawn to IncA *C. psittaci* protein (SEQ ID NO:8)and first method of use (vaccination).
Group VII, claims 5-12, drawn to IncA *C. trachomatis* protein (SEQ ID NO:14)and first method of use (vaccination).
Group VIII, claims 5-12, drawn to IncB *C. trachomatis* protein (SEQ ID NO:16)and first method of use (vaccination).
Group IX, claims 5-12, drawn to IncC *C. trachomatis* protein (SEQ ID NO:18)and first method of use (vaccination).
Group X, claims 13-17, drawn to a second method of use (detection of *Chlamydia*) of p242 *C. trachomatis* protein (SEQ ID NO:2).
Group XI, claims 13-17, drawn to a second method of use (detection of *Chlamydia*)of TroA *C. trachomatis* protein (SEQ ID NO:4).
Group XII, claims 13-17, drawn to a second method of use (detection of *Chlamydia*) of TroB *C. trachomatis* protein (SEQ ID NO:6).
Group XIII, claims 13-17, drawn to a second method of use (detection of *Chlamydia*)of IncA *C. psittaci* protein (SEQ ID NO:8).
Group XIV, claims 13-17, drawn to a second method of use (detection of *Chlamydia*)of IncB *C. psittaci* protein (SEQ ID NO:10).
Group XV, claims 13-17, drawn to a second method of use (detection of *Chlamydia*)of IncC *C. psittaci* protein (SEQ ID NO:12).
Group XVI, claims 13-17, drawn to a second method of use (detection of *Chlamydia*)of IncA *C. trachomatis* protein (SEQ ID NO:14).
Group XVII, claims 13-17, drawn to a second method of use (detection of *Chlamydia*)of IncB *C. trachomatis* protein (SEQ ID NO:16).
Group XVIII, claims 13-17, drawn to a second method of use (detection of *Chlamydia*)of IncC *C. trachomatis* protein (SEQ ID NO:18.

Group XIX, claim 18, drawn to a method of treatment of *Chlamydial* infection.

The inventions listed as Groups I-V do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:
Groups I-IX lack unity with each other as each group is drawn to a structurally (evidenced by different SEQ ID NO) and functionally distinct protein from two different microorganisms (*C. psittaci* and C. trachomatis).
Groups X-XVIII lack unity with each other as each group is drawn to a structurally (evidenced by different SEQ ID NO) and functionally distinct protein from two different microorganisms (*C. psittaci* and C. trachomatis).
Groups I-IX lack unity with Groups X-XVIII because Groups X-XVIII are claiming a second use for Groups I-IX.
Group XIX lacks unity with Groups I-IX because Group XIX is claiming a third use for the proteins of Groups I-IX.
Group XIX lacks unity with Groups X-XVIII because Group XIX is claiming a third use for the proteins of Groups X-XVIII.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/08744

Note that PCT Rule 13 does not provide for multiple products or methods within a single application. (See 37 CRF 1.475(d)).